# Mechanism of Selectivity of Diquat Solutions in Poppy.
## (*Papaver somniferum* L.)

by

**C.D.Barnes**

A thesis submitted in partial fulfilment of the requirements for the degree of

Bachelor of Agricultural Science with Honours

Department of Agricultural Science
University of Tasmania
Hobart

December 1993

# DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge, contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

C. D. Barnes

UNIVERSITY OF TASMANIA
HOBART

December, 1993

## ACKNOWLEDGMENTS

I wish firstly to sincerely thank my supervisor, Professor R.J. Clark, Head of Department of Agricultural Science, for his advice, constructive criticism and encouragement throughout this project, and also for being 'at hand' when needed.

Likewise, I extend my thanks to Dr. A.J. Fist of Tasmanian Alkaloids Pty. Ltd. for his guidance and enthusiasm throughout the duration of the project. I would also like to thank Messrs P.N. Matthews and P.A. Lowery for their assistance with the field trials, and for their advice on matters concerning the project.

I express my thanks to ICI Surface Science Laboratory, for the determination of dynamic surface tensions, and to Mr. P.Griffiths for his technical assistance regarding these measurements.

Appreciation is also extended to Mr A. Wells of Nufarm Pty. Ltd. for making available the emulsifiable concentrate used in the field trial.

I would like to thank the staff of the Central Science Laboratory, University of Tasmania for assistance with Scanning Electron Microscopy and Gas Chromatography-Mass Spectroscopy.

I wish to thank Dr N.J. Mendham, for his helpful discussion and recommendations on various aspects of this project. Appreciation is also extended to Dr. V. Dragar for her invaluable support with statistical analysis.

I express my thanks to Mr W. Peterson for his photography skills, and Mr. D. Bradford for his ability to 'produce the goods.'

Finally, to my family I say thank you, for without their encouragement and support all this would not have been possible.

# SUMMARY

The current mechanisms of herbicide selectivity in poppy (*Papaver somniferum* L.) were examined in glasshouse and field experiments.

Through measurements of contact angles of diquat solutions it was established that poppy, and the weeds fumitory, fat hen and field poppy are 'hard' to wet, whilst shepherds purse, wild radish, spear thistle and curled dock are relatively 'easy' to wet. It was revealed that diclofop-methyl has properties characteristic of a weak surfactant, compared with Agral®, a finding supported through measurements of dynamic surface tension.

The structure of the surface wax on the adaxial surface of poppy, field poppy, fumitory and fat hen leaves are described as seen under the scanning electron microscope. Chemical analysis of these waxes are also given. It appears the wax of fumitory and fat hen are influenced by application of ethofumesate.

Chemical analysis shows that ethofumesate can inhibit the deposition of primary alcohols, long chain aldehydes and alkanes on the developing leaves of fumitory and fat hen, making these plants more susceptible than poppy plants to future applications of diquat + diclofop-methyl.

Field trials conducted on the east and north-west of Tasmania, highlighted the difficulties that exist in extrapolating results from glasshouse experiments to field conditions. Measurements of spray retention and efficacy, revealed that plant responses to applications of diquat treatments were influenced, in addition to ethofumesate, by environmental conditions.

Plant responses at both sites demonstrated that the inert ingredients of the commercial formulation of diclofop-methyl, would, in admixture with diquat produce the same result compared with the current diquat/diclofop-methyl treatment.

# NOTES

Technical information about the herbicides and surfactants used in the study.

| Trade Name | Common Name | Chemical Description | Formulation Type |
|---|---|---|---|
| Agral | - | Nonyl phenyl ethylene oxide | 600 g/L condensate non-ionic organic surfactant |
| Asulox | Asulam | Methyl-4-sulfanilylcarbamate | 400 g/L aqueous concentrate |
| Hoegrass/Nugrass | Diclofop-methyl | 2-[4-(2-dichlorophenoxy) phenoxy]propanoate | 375 g/L emulsifiable concentrate |
| Newkalgen | - | ** a cationic | surfactant. |
| Reglone | Diquat | 6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinedium | 200 g/L aqueous solution |
| Tramat/Matrix | Ethofumesate | 2 Ethoxy-2,3 dihydro-3,3-dimethyl-5-benzofuranylmethane sulphonate | 500 g/L suspension concentrate |

** As yet has not been released commercially, therefore no chemical description or formulation type, apart from that given, is available.

# 1. Introduction

# 1. INTRODUCTION

From the first years of commercial poppy (*Papaver somniferum* L.) production in Tasmania, in 1969/70, it was recognised that poppies are poor competitors with weeds. If annual weeds such as wild radish (*Raphinus raphanistrum* L.), fumitory (*Fumaria muralis* Koch.) and shepherds purse (*Capsella bursa-pastoris* L.) are not adequately controlled yields are depressed through competition. Once established, other annual species such as fat hen (*Chenopodium album* L.), wireweed (*Polygonum aviculare* L.) and field poppy (*Papaver rhoeas* L.) also reduce yields through competition, but these weeds pose a further threat through their ability to interfere with harvesting and contaminate the harvested product. Thus, it was clear from the start of poppy production that the establishment of a clean crop is essential if maximum yields are to be realised. This philosophy and the spectrum of weeds encountered in poppy crops has changed little in over 20 years.

When the above mentioned weeds are present in a poppy crop a herbicide program commences with a tank mix of ethofumesate and asulam followed by a mixture of diquat and diclofop-methyl. The first spray is applied when the crop is at about the four to six leaf stage and the second spray about four days later. On reviewing the modes of action of each of these herbicides it is revealed that diclofop-methyl exhibits no phytotoxic effects towards dicotyledonous species. What role could this herbicide, which is registered for the control of annual grasses, play in a herbicide program designed for the selective control of dicotyledonous weed species? According to Fist (pers. comm. 1993) there appears to be a synergistic relationship between diquat and diclofop-methyl. If diclofop-methyl is not added diquat, which acts as a desiccant, will not kill the weeds. Although this spray mix does tend to retard crop growth slightly it is far less damaging to poppy plants than if a surfactant is used in place of diclofop-methyl. These observations indicate that a narrow margin of herbicide selectivity currently exists between crop and weeds. In an attempt to widen this selectivity many field experiments have been undertaken to find a suitable replacement for diclofop-methyl. However, there appear to have been no studies that have resulted in an understanding of why the diquat/diclofop-methyl mixture is selective.

Based on these observations this investigation sets out to examine the response of poppy and a range of weed species frequently encountered in poppy crops, to a number of diquat treatments applied prior to and after the conventional ethofumesate/asulam treatment. Consideration must be given to what effect the ethofumesate/asulam treatment, which is commonly referred to as the 'softening-up' spray, has on the efficacy of subsequent herbicide applications. Ethofumesate has been reported to inhibit leaf wax development on a number of plant species, (Leavitt *et al.*, 1978; Duncan *et al.*, 1981 and Duncan *et al.*, 1982) and it is possible that this 'softening-up' treatment is altering the wettability of weed and poppy leaves.

The physical nature of the diquat formulations, the wettability of leaf surfaces and leaf surface characteristics were determined in the laboratory. Determination of spray retention and efficacy were performed in the field. All of these measurements were made in an attempt to determine the extent to which differences in herbicide availability can explain the differences in species response seen in the field. This in turn should provide information that could facilitate the selection of a spray adjuvant to replace diclofop-methyl.

# 2. Literature Review

# 2. LITERATURE REVIEW

## 2.1. Introduction

### 2.1.1. History of Use and Cultivation

The oil or opium poppy (*Papaver somniferum*) has been known since antiquity (Krikorian *et al.*, 1975) and has been grown for at least 5,000 years (Lewis, 1977). It was probably first cultivated for the food value of its seed, which is rich in both oil and protein, but it was soon realised that extracts from other parts of the plant possessed peculiar narcotic properties (Bunting, 1963). The concentration of alkaloids in the latex of the unripe capsule was first recorded by Dioscorides at about A.D. 77 (Trease *et al.*, 1983), in which a distinction was made between the latex of the capsules, *opus*, and an extract of the whole plant, *mekonian*. Dioscorides also described a method for the production and harvesting of opium (the air dried latex of the capsule), a technique which persisted largely until the 1930's. This traditional method is labour intensive and as such only seed production proved economic in Western Europe, with the residues being burned or otherwise discarded (Bunting, 1963). Because of the increased pressures to provide opium alkaloids for an ever increasing world market, occasioned by better health care, attempts were made to exploit these residues as a source of morphine. With improvements in chemical processing this has been achieved. The extraction of the dried poppy capsule is currently replacing opium collection throughout the world as the source of morphine alkaloids.

The United Nations Opium Conference Protocol which limits and regulates the cultivation of *P. somniferum* asserts that Bulgaria, Czechoslovakia, Hungary, India, Iran, Pakistan, Tasmania, Turkey, and certain states of the former U.S.S.R and Yugoslavia are the only countries that may legally produce opium. However, countries other than these such as the Netherlands and Poland cultivate *P. somniferum* exclusively for seed or oil.

### 2.1.2. Cultivation of *P.somniferum* in Australia

As early as 1891, the benefits of commercial poppy production in Australia were recognised. With imports far outweighing exports at the time Turner (1891) suggested "Farmers in this Colony .... might do much worse than put under cultivation an acre or two of the opium poppy. It is by attending to these 'small cultures' that farmers can ever hope to make their calling a more lucrative one." Yet, little was done until the Department of Agriculture in collaboration with the Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O) decided to perform trials with poppies during the Second World War years, 1941-1944 (Loftus-Hills, 1946). In 1958 representatives of Messrs T. and H. Smith Ltd, a Scottish pharmaceutical manufacturing and supply firm explored poppy production possibilities in Australia.

The selection of Tasmania followed four years of trials in and around Australia. In the years 1960-64 trials were undertaken in N.S.W, S.A, W.A, N.Z and Tasmania in regions where good climatic conditions had been identified (Davies, 1985). The combination of suitable climatic and soil conditions; namely spring rains with high sunshine hours and hot dry summers, and well drained fertile soils with high organic matter and a pH no less than 5.7 culminated in future trials being restricted to Tasmania. This led to a Commonwealth Agricultural Council agreement amongst the Australian States restricting poppy production to Tasmania. Large scale production commenced in the 1969/70 season when 297 ha were sown (Walker, 1977). The area on which poppies are grown is now in the order of 8000 ha and Tasmania supplies about 40% of the world opiate market (Fist, pers. comm. 1993).

## 2.2. Morphology

*P.somniferum* is an annual herb which belongs to the *Papaveraceae* family (Curtis and Morris, 1981). Hyde-Wyatt and Morris (1975) present the following description of the plants development; the cotyledon is sessile, 10 to 15 mm long and hairless. The first two leaves appear as a pair, but subsequent leaves grow singly. The first two leaves are 8 to 15 mm long overall of which rather less than half is petiole, they have simple margins and the next two to three have small lobes. Thereafter, the leaves have lobes which become larger and more numerous. The lower leaves are shortly stalked, the upper sessile and stem glasping, glaucous, glabrous or with some stiff hairs. The stem leaves are 80 to 150 mm long, while towards the top of the stem the leaves are smaller. The plant develops as a rosette which tends to have leaves semi-erect rather than flat.

The mature plant is erect in habit with stems which may be branched and reaches a height of 1.4m. In cultivation this species is variable in height depending on the fertility status of the soil and the time of year when germinated. The stem is solid and pithy, fluted in cross-section, and is hairless or has only a few hairs.

The flower is terminal and single, some 50 to 80 mm in diameter. The four petals are lilac and usually have a darker basal blotch. The capsule is more or less spherical, 20 to 40 mm in diameter with a flat plate like cap.

A diagrammatic representation of the plant is shown in Figure 2.1; (extracted from Hyde-Wyatt and Morris, 1975).

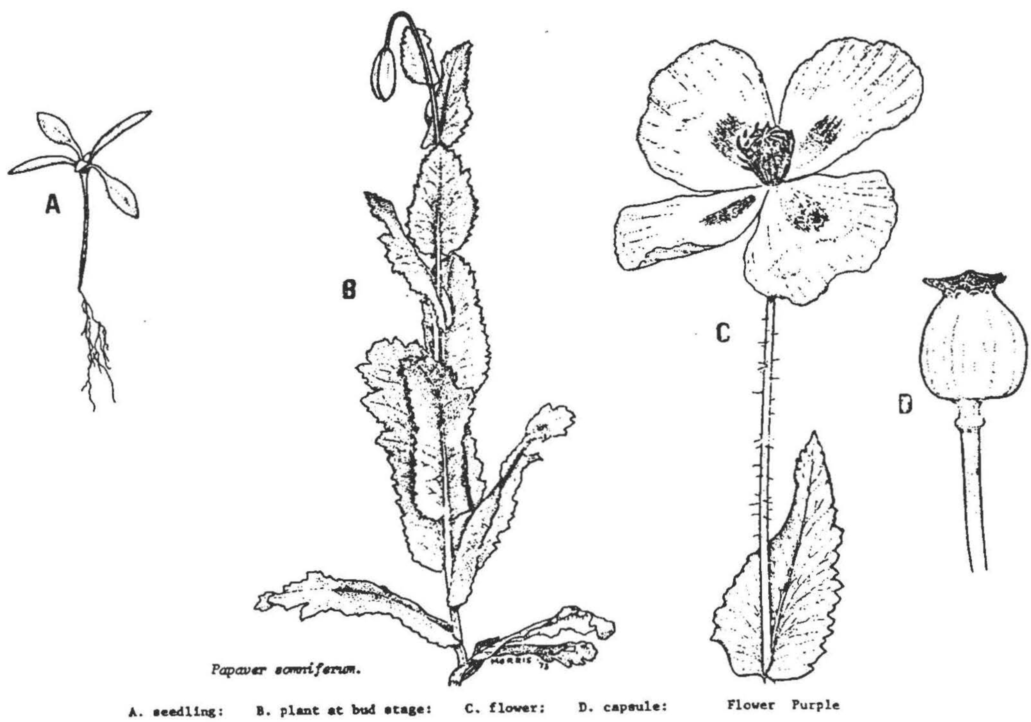



Figure 2.1: Diagrammatic representation of poppy plant. (Adapted from Hyde-Wyatt and Morris, 1975.)

## 2.3. History of Weed Control

Weeds have challenged man's effort to survive ever since the soil was tilled for food. During thousands of years this challenge has been meet, and in the process produced many amazing advances. First a sharp stick was substituted for fingers. Centuries later the metal hoe was discovered. Labour was then reduced by harnessing a horse or ox to drag the hoe or plough. In 1731 a major advance was proposed by Jethro Tull-planting crops in rows to permit "horse-hoeing" (cited in Klingman and Ashton, 1982). Less than 200 years later tractors started to replace horses.

At about the time of Jethro Tull's proposal the benefit crop rotations conferred to weed control was being realised. The rotation, traditionally concerned with the maintenance of soil fertility and the control of soil diseases, soon had weed control as one of its major objectives.

Each of these activities did much to reduce the number of weeds able to become established and set seed. Yet, they were not enough to ensure a seed crop was free from weeds. The cleaning of seed by movement of air, winnowing, dates from ancient times (Hance and Holly, 1990). By the 18th Century manually operated hand winnowers were wide spread, and later sieves and screens were incorporated to exploit the physical differences between crop and weed seeds. Since the introduction of machinery in the 1920's increasingly sophisticated methods of seed cleaning have been developed (eg gravity and electrostatic seperators).

All these methods battle weeds with force, but with the development of herbicides, chemical energy has replaced this mechanical energy for weed control.

Chemical weedkilling originated in 1896 when Bonnett, a French grape grower, observed that the Bordeaux mixture, (a mix of copper sulphate and lime) he applied to his vines as a protection against downy mildew, turned the leaves of yellow charlock (*Sinapsis arvensis* L.) black. The weedkilling properties of sulphates of ammonia, zinc, iron, and other metals were then soon observed (Brian, 1976).

A later milestone in weed control was the introduction of the first organic chemical, 2-methyl-4,6-dinitrophenol (DNOC) in 1932 (Brian, 1964). Here at last, was the first chemical replacement for the hoe. Chemical weed control, however, developed rapidly only after the discovery in 1941 that the salts of the chlorinated phenoxy acetic acids were selectively herbicidal (Hance and Holly, 1990). Since then industrial research has led to the release of a wide range of non-selective and, later selective residual and non-residual herbicides.

It is through these improvements in agricultural technology that man has been able to progressively produce more food. For example, in 10,000 B.C when weeds were removed from crops by hand, one person could hardly feed himself and starvation was common. Yet by 1980, with weeds being controlled predominantly through the use of herbicides, one farmer could feed 38 persons (Klingman and Ashton, 1982).

### 2.3.1. History of Surfactants

Gillette was one of the first researchers to study the effect of surfactants and in 1887 he reported on the use of kerosene and soap solutions to destroy insect eggs (cited in McWhorter, 1982). Many reports soon followed on the use of surfactants in insecticide and fungicide solutions, but little attention was given to the importance of surfactants in herbicide solutions until the introduction of the synthetic organic herbicides in the late 1930's.

It was still some time before investigators realised significant increases in the activity of herbicides could be achieved from the addition of surfactants. Few investigators studied the influence of surfactant concentration, and furthermore the same surfactant was seldom used in work with different herbicides (McWhorter, 1982). Nevertheless, by the 1950's the advantages which surfactants had on spray solutions were clearly recognised, but agricultural surfactants were not available so household detergents were recommended.

Research on the use of surfactants with herbicides greatly increased in the 1950's. The discovery by McWhorter (1955; cited in McWhorter, 1982) that the addition of paraffinic oils to s-triazine herbicides increased herbicide effectiveness was impetus to surfactant use. By the 1960's a vast amount of additional research on surfactants had taken place and wide-scale farmer acceptance of their use soon followed.

## 2.4. Weed control in poppies. Past and present

In the first years of commercial production of poppies in Tasmania it was realised that poppies are poor competitors with weeds, and the establishment of a clean crop is essential to achieve maximum yields (Allen and Frappell, 1970). Early methods of weed control relied on the provision of a clean seed bed, followed by mechanical weeding with inter-row equipment and cross harrowing. Although these mechanical methods had been successful for controlling weeds in European poppy crops, they were unsuited to the clay krasnozems of the North West Coast (Davies, 1985). The weeds within rows were not controlled and these reduced yields through competition with the crop; other weeds, such as fat hen interfered with harvesting and contaminated the harvested products (Baldwin, 1976).

The spectrum of weeds encountered in poppy crops can be broadly classified as either a) annual b) perennial or c) biennial species. Annual weeds, such as wild radish, fumitory, wireweed, shepherds purse, fat hen, and field poppy are frequently found in poppy crops. Due to their potential to reduce yields through competition and contamination they pose a significant threat to most growers. The perennial; curled dock (*Rumex crispus* L.), and the biennial; spear thistle (*Cirsium vulgare* Savi.), weeds are less common, however, when not controlled they also adversely affect yields.

As no information was available on chemical weed control in poppies, a program of herbicide assessment was initiated in 1965. Of 41 herbicides screened at the time only diquat, nitrofen and flurodifen showed any selective properties (Baldwin, 1976).

Field experiments conducted on the North West Coast by Baldwin (1976), found that diquat (Reglone®) and nitrofen (Tok-E-25®) could be used for selective weed control in oil seed poppy. Various mixtures of diquat and nitrofen applied sequentially at different rates and timing, dependent upon weed spectrum, crop stage, soil type and moisture, provided weed control until 1979. It was an extremely subjective basis requiring great skill. Weed control especially of fat hen and wireweed was often inadequate (Matthews, pers. comm. 1993). During the late 1970's Hans Klass of Schering PTY. LTD. determined that barban (Neoban®) performed similarly to nitrofen when mixed with diquat. Barban was to later replace nitrofen, as in 1979 nitrofen was removed from the market for toxicological reasons (Shaw, 1985).

By the early 1980's weed control consisted of two treatments; firstly a mixture of asulam (Asulox®) and ethofumesate (Tramat®) followed by diquat and barban. Asulam had previously been used alone to control docks and to a lesser extent cruciferous weeds. Weed control was considerably improved by this development although it still remained subjective and continued to require skill, especially in less than optimal conditions.

During the mid 1980's diclofop-methyl (Hoegrass®) was identified as an acceptable adjunct to diquat. This formulation appeared to be less severe on poppies in circumstances where soil moisture was less than optimal. In 1990, the production of barban ceased and weed control since that time has been dependent on diclofop-methyl (Matthews, pers. comm. 1993).

In addition to the standard asulam/ethofumesate and diquat/diclofop-methyl treatment fluroxypyr (Starane®) is used for control of volunteer potatoes, (for which it is registered) and a number of other broadleaf weeds. Diflufenican (Brodal®) is effective on all the crucifers although it is poorly tolerated by poppies in less than ideal conditions.

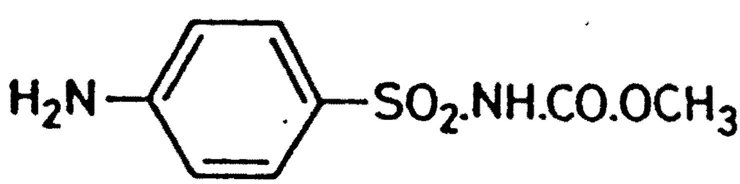
The herbicides sethoxydim (Sertin®), fluazifop (Fusilade®) and quizalofop-p-ethyl (Targa®) have all been successfully used on poppies in appropriate circumstances (Matthews, pers. comm. 1993).

As illustrated by this introduction, weed control in poppy crops has progressed a long way since the initial reliance on mechanical methods. In association with these improvements there have been corresponding increases in yield and quality of the harvested product. But how effective is the current chemical weed control strategy? It is only after one has examined the modes of action of each of the principle herbicides used in weed control and explored those factors which affect the efficacy of these herbicides (eg formulation, trajectory and retention) can this, and other questions raised in the course of the discussion be answered.

### 2.4.1. Mode of action of herbicides currently used in weed control

**2.4.1.a** Asulam A carbamate herbicide, the structure of which is presented in Figure 2.2. The trade name is Asulox®.

Fig 2.2



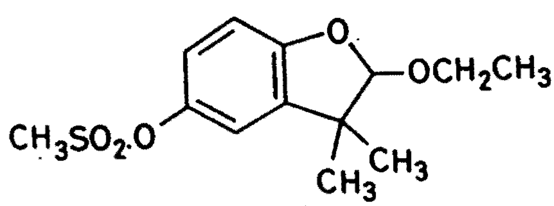

Methyl 4-sulfanilylcarbamate

The biological activity of the carbamate compounds has been known since 1929 when Friesan showed that phenylurethane retarded germination of oats and wheat (cited in Brian, 1976). The selective nature of these herbicides was announced by Templeman and Seaton (1945, cited in Brian, 1976), after they observed that monocotyledon cereal species were more susceptible than dicotyledonous species to carbamate herbicides.

The selectivity of asulam was first recorded by Cottrell and Heywood (1965, cited in Brian, 1976). The herbicide is readily absorbed by susceptible species and once absorbed is translocated primarily in the symplast (Klingman and Ashton, 1982). Its action appears to involve an inhibition of ribonucleic acid and protein synthesis, and although it does not significantly reduce photosynthesis, asulam does inhibit bud growth (Ashton and Crafts, 1981).

**2.4.1.b** Ethofumesate A benzofuran compound developed by the Schering Chemical Company in 1974 (Thomson, 1989). The chemical structure is presented in Figure 2.3. The trade names are Tramat® and Matrix®.

Figure 2.3



2.1, Ethoxy-2,3 dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate

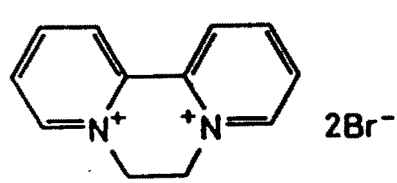
Selectivity of ethofumesate is dependent upon its absorption and translocation. Working with sugar beet (*Beta vulgaris* L.), Duncan *et al.*, (1981) found that absorption of foliar applied ethofumesate is dependent on stage of growth at treatment. Susceptible species, such as fat hen absorbing considerably more ethofumesate at both the two and four leaf stages of growth than tolerant sugar beet. Tolerant species are able to endure treatments of ethofumesate because there is also no accumulation of the compound in untreated plant segments. Yet, in susceptible species ethofumesate is translocated acropetally to untreated leaf tissue.

Duncan *et al.*, (1981) reported that photosynthesis and dark respiration are strongly inhibited by ethofumesate. Inhibition is followed by a rapid recovery in tolerant species, yet not in susceptible species. Thus, a rapid and extensive absorption of ethofumesate, an extensive accumulation in untreated plant components, slowed metabolism and inhibited photosynthesis all play an active role in the susceptibility of a given plant species to foliar applied ethofumesate.

Inhibition of epicuticular wax deposition is an indirect effect of ethofumesate, first investigated by Leavitt *et al.*, (1978) following observations that ethofumesate treated sugar beet emerged with glossy leaves. As early as 1956, Dewey *et al.*, (cited in Leavitt *et al.*, 1978) reported that such a decrease in wax deposition increased transpiration rates and spray retention, which in turn increases a plant's susceptibility to subsequent foliar applied herbicides. The ramifications of this are discussed later in regard to the composition of plant epicuticular waxes.

**2.4.1.c** Diquat A heterocyclic organic compound which belongs to the bipyridylium quaternary ammonium class. The trade name is Reglone®. The phytotoxic properties of the compound were realised in 1955 by the Imperial Chemical Industries of England (Brian, 1976). The chemical structure is provide in Figure 2.4

Figure 2.4.




6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinedium

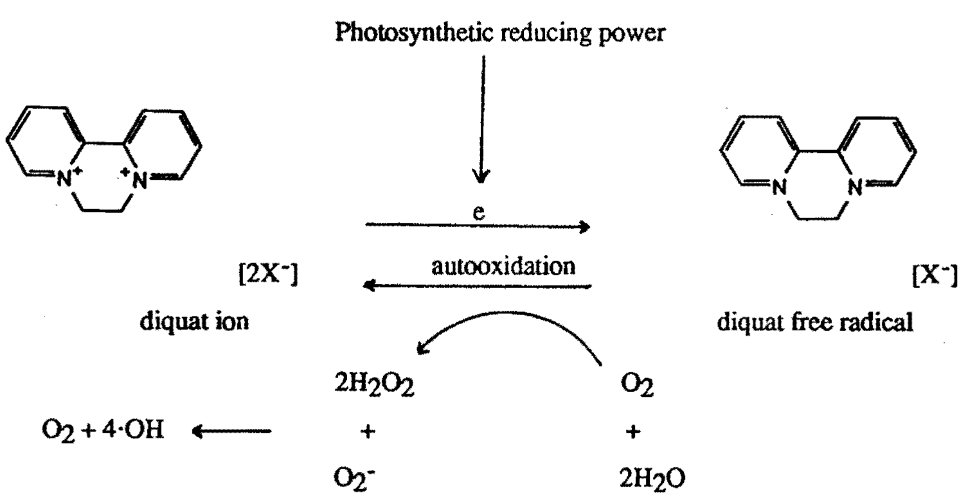
Upon application diquat causes wilting and rapid desiccation of foliage of susceptible plants, often within a few hours. Best results in the field have been obtained by a late afternoon, rather than a morning or mid-day application (Fist, pers. comm. 1993). This appears to allow some internal transport during the night, before acute phytotoxicity induced by light, which could limit movement.

Klingman and Ashton (1982) reported that translocation after foliar application appears to be almost solely via the apoplastic system. However, after the loss of membrane integrity, diquat moves into untreated leaves, presumably along with the flow of other cellular contents (Klingman and Ashton, 1982).

Diquat itself is not biologically active, and only becomes so on the reversible conversion from the ion form to the free radical form (Klingman and Ashton, 1982). This interconversion, as outlined in Figure 2.5 is cyclic and requires light, molecular oxygen, water, and the photosynthetic apparatus.

Figure 2.5

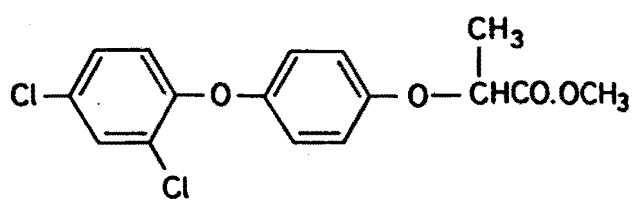
*Mode of Action of Diquat*




According to Klingman and Ashton (1982) during autoxidation of the diquat free radical to the ion, four by-products are formed, namely: (1) $H_2O_2$ (hydrogen peroxide), (2) $O_2^-$ (superoxide radical), (3) OH (hydroxyl radical) and (4) $O_2$ (singlet oxygen). Each of these products are potentially phytotoxic yet, Brian (1976) believes that the hydroxyl radical and the superoxide radical are responsible for the phytotoxic symptoms.

**2.4.1.d** **Diclofop-methyl** This compound belongs to the polycyclic alkanoic group of herbicides (Andrews, 1990). The trade names are Hoegrass® and Nugrass®. The chemical structure is presented in Figure 2.6.

Figure 2.6




2.1.1.(2.dichlorophenoxy)phenoxylpropanoate

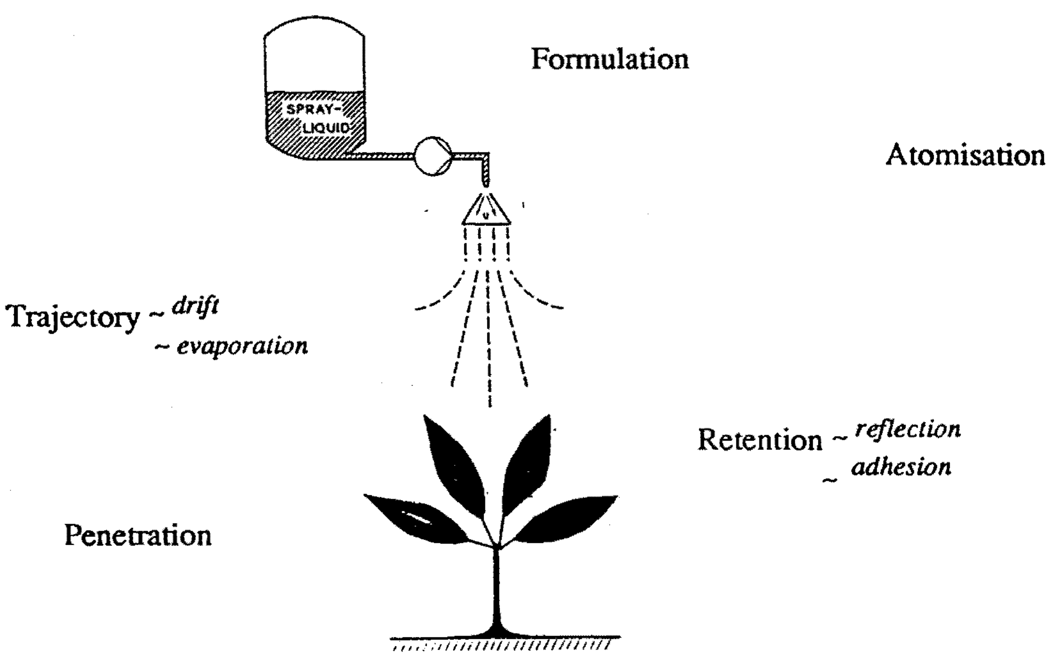
Diclofop-methyl is registered as a selective, post-emergence herbicide for the control of wild oat and other annual grasses in lupins, peas, wheat, barley, triticale, rapeseed, cereal rye, linseed, and safflower (Chambers, 1993). The difference in selectivity is due to differential metabolism between tolerant and susceptible species. Diclofop-methyl undergoes demethylation to form diclofop, which undergoes ring hydroxylation in tolerant species; this hydroxylated form is non toxic (Hance and Holly, 1990). In susceptible grass species diclofop-methyl forms a diclofop conjugate through an ester linkage. This phytotoxic diclofop is released, inhibiting acetyl-coenzyme A carboxylase, a key enzyme in the biosynthesis of fatty acids essential for membrane synthesis and renewal (Andrews, 1990).

With only this information in mind one would be doubtful of the ability of diclofop-methyl to bestow any advantage to broad leaf weed control in poppy crops. Yet, whenever herbicide combinations are used potential for interaction exists. Such an interaction occurs in the diquat/diclofop-methyl mixture. Diclofop-methyl has been suspected to act as a selective wetting agent in diquat solutions; (Fist, pers. comm. 1993), and is an integral component of the current weed control program used in poppy crops. As yet, no experimental evidence exists to support the claim that diclofop-methyl acts as a wetter.

## 2.5. Factors Affecting Efficiency of Foliar Applied Herbicides

The transportation of the active ingredient of a herbicide commences with the preparation of the spray solution and is followed by atomisation, trajectory and impact on the leaf surface. Foliar penetration and subsequent translocation within the plant tissue are also important for systemically acting compounds (Wirth *et al.*, 1991). Each of these steps, as outlined in Figure 2.7 must be optimised to obtain maximum efficiency from a spray application, whilst minimising the risk to the environment.

Figure 2.7 Factors affecting efficiency of foliar applied herbicides




This section deals with each one of these processes and the manner by which they affect herbicide application in *Papaver somniferum*.

## 2.6. Formulation

It was apparent by the late 1950's that the efficiency of herbicides; and other pesticides for that matter, may be considerably influenced by the manner in which they are formulated for use. The efficiency being determined by the ability of the formulation to meet the following requirements.

### 2.6.1. Physico-chemical requirements

The solubility of the herbicide in water and organic solvents is the main parameter influencing the choice of formulation type. The major types being as follows:

**2.6.1.a** **Soluble powders and solutions** Many herbicides are insoluble in water. However, their salts may be very soluble. For example, asulam sodium salt is more than 40% soluble, whilst the free acid is only 0.5% soluble (Foden, 1972). The sodium and amine salts of 2,4-D, MCPA, TCA and dinoseb are also soluble in water. These salts, and a select number of readily soluble compounds, such as diquat are simply formulated as aqueous solutions.

**2.6.1.b** **Emulsifiable concentrates** A herbicide which is insoluble in water may be sufficiently soluble in an organic solvent to be formulated as an emulsifiable concentrate. The emulsifiable concentrate most commonly formulated as an *oil-in-water* (O/W) emulsion; the term 'oil' used to denote the water insoluble fluid (Adamson, 1982). For this purpose it is important that the chosen solvent is itself insoluble in water to avoid precipitation of the active ingredient when the formulation is added to water. Solvents used in the formulation of phenoxy-alkanoic ester herbicides include normal and isomeric paraffins. Those compounds not soluble in paraffinic solvents may be formulated in petroleum-derived aromatic solvents which are generally capable of dissolving much greater concentrations of herbicide (eg xylene, kerosene, toluene), (Foden, 1972).

**2.6.1.c** **Hydrophilic-Lipophilic Balance (HLB)** The preparation of an emulsifiable concentrate formulation from a simple solution of herbicide in an organic solvent requires the addition of an emulsifying agent. The process of selecting a suitable agent is often difficult given that in 1971, McCutcheon (cited in Foden, 1972) listed approximately four thousand emulsifying agents, the majority of which could be used as emulsifiers in herbicide formulations. Basing one's choice on formulation stability, it is essential that the favoured emulsifier be adsorbed at the oil/water interface. Once adsorbed it must be orientated so that the hydrophobic portion of the molecule is contained in the oil phase, its polar or hydrophilic portion being in the water phase. The balance between the hydrophobic and hydrophilic portion of the molecule controlling the efficiency of the emulsifying agent (Furmidge, 1959). The importance of this balance led to the conception of the hydrophile-lipophile balance (HLB) by Griffin (1949, cited in Osipow, 1962). Emulsifiers are classified according to the size and the strength of the hydrophilic and lipophilic portion of the molecule. The balance of these two opposing groups forming the HLB, the scale of which is outlined in Table 2.1

Table 2.1. The HLB Scale

| Surfactant solubility behavior in water | HLB number | | Application |
|---|---|---|---|
| No dispersibility in water | 0, 2 | | |
| | 2, 4 | | W/O emulsifier |
| Poor dispersibility | 6 | | |
| Milky dispersion; unstable | 8 | | Wetting agent |
| Milky dispersion; stable | 10 | | |
| Translucent to clear solution | 12, 14 | Detergent | O/W emulsifier |
| Clear solution | 14, 16, 18 | Solubilizer | |

According to this system, an emulsifier that is lipophilic in character is assigned a low HLB number, while an emulsifier that is hydrophobic in character is assigned a high number. The concept has come into fairly wide use due to its ability to facilitate the selection of a suitable emulsifying agent .

**2.6.1.d** **Wettable powders** Some herbicides are soluble neither in water nor in acceptable organic solvents. Generally such intractable compounds are formulated as wettable powders in which the active ingredient is finely divided by milling and combined with a powdered carrier. An alternative means of dealing with insoluble herbicides is to formulate them as emulsifiable or dispersable suspension concentrates, or 'flowables' as they are often called.

**2.6.1.e** **Suspension concentrates** In suspension concentrates the finely divided herbicide is dispersed in a fluid medium, which may be water or an oil. Aqueous suspension concentrates form simple dispersions of the herbicide on addition to water. Oil based suspensions form oil-in-water emulsions in the manner of emulsifiable concentrates, except that the herbicide is suspended rather than dissolved in the oil phase. In addition, it is essential to structure or thicken the suspension to overcome gravitational settling of the herbicide (Foden, 1972).

Other formulation styles include: multiple emulsions, microemulsions, liquid crystals, suspension emulsions and water dispersable granules (Griffiths, pers. comm. 1993).

Clearly the choice of herbicide formulation is dictated largely by the physico-chemical properties of the herbicide, yet equal consideration must be given to use and biological requirements.

### 2.6.2. Use Requirements

The composition of a herbicide formulation is influenced to a greater or lesser degree by cost, storage, and ease of application.

Cost and manufacturing requirements may dictate some detailed modification of the formulation. For example, it may be necessary to replace obsolete or expensive emulsifiers or other formulation components.

The formulation must have an adequate shelf-life, even when subject to adverse environmental conditions. Normally this has to be two years or more because of the seasonal nature of the business and the need for stock to be carried over to the following season if unsold or unused by the farmer.

The formulation must also be able to manage dilution into water with a wide range of hardness, composition and temperature, frequently in admixture with other pesticides. The diluted composition must maintain itself in suspension or solution for a period of hours or even days through a variety of sprayers, which may or may not be agitated. The dilution factor may range from 1:5 to 1:1000, and in many cases the same formulation has to cope with these extremes (Seamen, 1979).

Finally there are non-herbicidal use requirements to be met by the formulation. The most important is that of safety to the user and to the environment and public. Before the formulated product is released onto the market it is thoroughly tested to ensure that it is effective as well as safe. These studies examine :

- efficacy.
- toxicology; acute and chronic.
- determination of residue status in food and feed crops.
- fate in the environment (residues in soil, runoff, groundwater and wildlife).
- impact on the environment (induced changes in natural populations of animals, plants and soil microorganisms).
- biodegradibilty.

The research examines not only the applied component, but also breakdown products. As a result we can be assured that when applied according to label instructions the herbicide formulation is safe to use.

### 2.6.3. Biological Requirements

Of all the components which affect the activity of a herbicide formulation, the water soluble surface-active agents; commonly called adjuvants, wetters, dispersants or surfactants, have received the greatest attention. These substances, (hereinafter referred to as surfactants) are defined by the Weed Science Society of America (WSSA) as materials "...that facilitate and accentuate the emulsifying, dispersing, spreading, wetting or other surface-modifying properties of liquids" (McWhorter, 1982).

#### 2.6.3.a Classes of Surfactants

Surfactants are commonly classified as anionic, cationic or non-ionic depending on the nature of the electrical charge, or absence of ionisation on the hydrophilic portion of the molecule (Parr and Norman, 1965).

**Anionic Surfactants** Compounds having a hydrophobic group (paraffinc chain, alkyl-substituted benzene or naphthalene ring) balanced with a negatively charged hydrophobic group (carboxyl, sulfate or phosphate).

**Cationic Surfactants** Compounds having similar hydrophobic groups as listed for anionic surfactants, but balanced with a positively charged hydrophobic group (quaternary ammonium, sulfonium, or phosphonium).

**Non-ionic Surfactants** Compounds characterised by the absence of an ionised group. The hydrophobic group is balanced by such non-ionised hydrophilic groups as polymerised ethylene oxide. As this ethylene oxide content of a surfactant is raised the molecule naturally becomes more hydrophilic and hence more water soluble. According to Nelson and Garlich (1969) such an increase in the hydrophile-lipophile balance (HLB), raises herbicidal activity. In contrast to this, Norris (1973) found the surfactant effect to be inversely related to the HLB. Chow and Taylor (1980) altered the HLB of nonyl phenol ethylene oxide condensates and found that although the surfactants enhanced herbicide activity, there was no correlation between herbicide efficacy and the HLB of the added surfactant. The earlier results had led Hull *et al.*, (1975) to conclude that the exact effect of the HLB of a surfactant, in regards to herbicide efficacy is difficult to forecast. This is because it can be markedly altered by complex interactions of the physical and chemical nature of the active ingredient, the type of carrier and the nature of the leaf surface in question. It is, therefore, not the HLB used in the selection of a surfactant for a specific application, but rather the critical micelle concentration (Singh *et al.*, 1984).

### 2.6.3.b Micelle Formation

Surfactants have minimal water stability and at relatively low concentrations (say 0.02%) individual molecules exist in hydrated form in solution. When the surfactant concentration exceeds a critical level in an aqueous system, the molecules do not precipitate but aggregate into clusters called micelles. This critical micelle concentration (c.m.c), as reported by Furmidge (1959), Osipow (1962), Parr and Norman (1965), Singh *et al.*, (1984) is associated with abrupt changes in many characteristic properties of the surfactant. The maximum potential of the surfactant for lowering the surface tension and the interfacial tension of an aqueous solution is reached in the c.m.c range (see Figure 2.8). Singh *et al.*, (1984) found that after the c.m.c is reached a decrease in surface and interfacial tension is minimal. This is because as surfactant concentration is increased there is a corresponding increase in osmotic pressure until a plateau is reached in the c.m.c range, beyond which little change occurs.

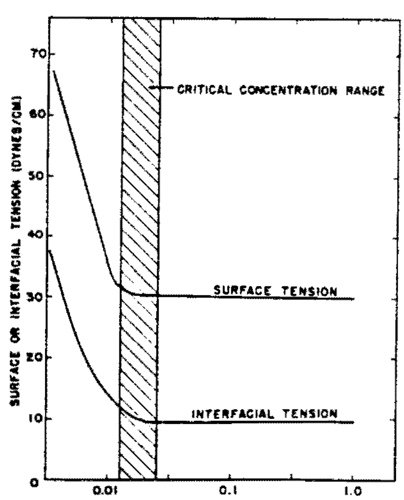



Figure 2.8. Effect of increasing concentration of a typical non-ionic surfactant on surface and interfacial tension of aqueous system. (From Parr and Norman, 1965).

According to Parr and Norman (1965) and Foden (1972) the c.m.c for most surfactants is in the region of 0.01 % to 0.1%; however, concentrations as high as 0.2% or considerably lower than 0.001% are not uncommon.

#### 2.6.3.c Surface Properties

The surface of any given liquid behaves differently than the internal phase of that liquid. This is because a molecule in the bulk phase of a liquid is completely surrounded by other molecules. However, on the surface of the liquid the molecules do not have neighbouring molecules of that bulk phase on one side. This produces an imbalance of energy resulting in a 'skin effect', or surface tension of the liquid. Thus, surface tension is related to an excess energy localised on the surface.

This liquid surface tension is that which a drop presents to the solid leaf surface on impacting. If the drop does not include a surfactant the surface tension is that of the pure liquid from which it is derived (often referred to as the static, final or equilibrium surface tension). If, however, the liquid contains a surfactant the surface tension varies with the age of the drop as adsorption of the surfactant to the liquid surface takes place (Mysels, 1986). This changing surface tension is referred to as the dynamic surface tension (DST).

It is now accepted that the DST of the herbicide formulation gives a better correlation with retention than the equilibrium surface tension (De Ruiter *et al.*, 1990; Grayson *et al.*, 1991; Wirth *et al.*, 1991). Grayson *et al.*, (1990) declaring that "...equilibrium surface tensions of solutions containing surfactants are misleading and that better correlations can be obtained with dynamic surface tensions."

## 2.7. Atomisation and Trajectory

The conversion of a bulk of the spray formulation into droplets can be achieved using several forms of atomisers or nozzles. When herbicides are applied with ground equipment, fan type nozzles are generally used to apply carrier volumes of

140 l/ha or greater. Cone pattern nozzles are routinely used to apply insecticides and fungicides on foliage, but rarely to apply herbicides (Reichard and Triplett, 1983). Apart from these hydraulic atomising nozzles, rotary atomisers have also been developed and marketed for agricultural use. By either method of atomisation, droplets are produced, in effect, by disintegration of thin liquid sheets.

### 2.7.1. Atomiser Selection

Atomiser selection can influence weed control, as was demonstrated by Reichard and Triplett (1983). They found that fan pattern atomisers were more effective in controlling weeds with paraquat (1,1'-dimethyl-4,4'-bypyridinium ion) than rotary atomisers. The flat fan nozzles provided a more consistent coverage regardless of pressure whilst differences in coverage, commonly due to the production of a wide drop size range, and subsequent efficacy existed in rotary and flood nozzles. The angle of the atomiser was also found to influence herbicide efficacy, with those atomisers with spray angles less than 80° increasing penetration into the canopy. The reason being that the probability of a droplet being intercepted near the top of the foliage is greater for a droplet that is projected with a nearly horizontal rather than a vertical trajectory. Obviously this affect will be dependent on the nature of the foliage of those plants which make up the canopy.

### 2.7.2. Drop Size

A range of nozzles and operating pressures are available for herbicide application, and any combination of the two produces a differing atomising capacity. It is this capacity for droplet production which regulates the volume mean diameter (VMD) of the spray (the VMD being the average drop size calculated on a volume basis). In general, droplet size as measured by VMD, decreases with an in increase in pressure while increases as the size of the atomiser increases or as the dynamic surface tension of the liquid increases. Work by Brunskill (1956, cited in Holly, 1976), Hartley and Brunskill (1957), and McKinlay *et al.*, (1974) all show that droplet size has a profound effect on spray retention and in turn spray efficacy. Brunskill (1956, cited in Holly, 1976) studied the behaviour of individual droplets impinging on pea (*Pisum sativum* L.) leaves and observed that retention was high below a certain critical droplet diameter and was low, falling nearly to zero, at large droplet diameters. This critical diameter in the case of water on pea leaves is ~ 100 μm, and according to Holly (1976) it increases as the DST of the formulation decreases. Inevitably the critical droplet diameter will vary from species to species which will produce retention differences and hence effect selectivity. This was demonstrated by results obtained with paraquat and diquat by Douglas (1968), in which maximum kill of wireweed was given by droplets of 250 μm diameter whereas damage to bean (*Phaseolus vulgaris* L.) plants increased with droplet size up to 400-500 μm.

In selecting a suitable drop size for herbicide application one must consider that in the field, droplet size inevitably influences both drift and evaporation during trajection. McKinlay *et al.*, (1974) found that laboratory applied sprays of smaller drops (100 μm) were more phytotoxic than sprays of the same dosage level made up of larger droplets (350 μm). Reed *et al.*, (1990) investigated the efficacy of tridiphane plus atrazine applied postemergence to field corn (*Zea mays* L.) and found that drift and faster drying of smaller droplets impeded herbicide performance. In earlier work Douglas (1968) proposed that the droplet spectrum of the bipyridyl herbicides be limited to a minimum droplet size of 250 μm to avoid a major drift hazard. It was proposed that the upper limit be defined in individual plant tests as larger droplets tend to be reflected or to coalesce and run off leaf surfaces more readily than smaller drops.

### 2.7.3. Spray Volume and Concentration

The effect of drop size on herbicide efficacy must not be considered in isolation, since the volume of dilutent used also effects plant coverage and potentially the phytotoxicity of the spray. The amount of active ingredient applied per unit area will have a marked effect also, and would be expected to interact with drop size and volume.

McKinlay *et al.*, (1974) investigated the interaction of drop size, spray volume and concentration and its subsequent effects on paraquat toxicity using sunflower (*Helianthus annuus* L.). Under laboratory conditions it was found that at low dosage rates of paraquat (35 g/ha), low carrier volumes (5.5 L/ha) were significantly more effective than high spray volumes (22 L/ha) when 100 μm drops were used. It was suggested that penetration into the leaf was faster from the more concentrated solution, although there was greater plant coverage with the higher volume. At a higher dosage rate (140 g/ha), spray volume was found to have no effect on toxicity with 100 μm drops, yet, the higher spray volume was more effective than the lower when the 140 g/ha was applied in 350 μm drops. Unfortunately, in providing these results, no account has been made for differences associated with laboratory and field conditions (eg drift of smaller drops). It should also be noted that the carrier volumes chosen are very low, and according to Reichard and Triplett (1983) such low carrier volumes would not consistently control vegetation because of poor foliar coverage.

## 2.8. Retention

Over sixty five years ago Aslander (1927, cited in Holly, 1976) concluded that the effectiveness of dilute sulphuric acid as a selective herbicide for cereal crops, was dependent on the differential retention of spray droplets. Following this, Blackman and Templeman (1936, cited in Holly, 1976) established that retention was dependent both on the morphological characteristics of the shoot and the physical properties of the spray.

Since that time there have been many studies on the retention of sprays by plants. It is the aim of this section to deal with retention of spray solutions by plant surfaces, which will bring together those earlier discussed variables which are in the control of the applicator (eg formulation and atomiser selection).

### 2.8.1. The effect of physical characteristics of the spray on retention.

Furmidge (1959) considered a spray droplet as an elastic sphere which, at the moment of impact possess kinetic energy, some of which is transmitted to the surface on which impaction takes place and most of the remainder is absorbed initially as compressive strain energy. This is transformed into (a) kinetic energy which causes the droplet to flatten radially from the point of impact, and (b) surface energy which increases as the droplet flattens owing to the increase in the surface area of the two interfaces, liquid-air and liquid-solid. The resultant forces on the droplet will be partially in opposition and the fate of the droplet will depend largely upon the point at which equilibrium is reached.

**2.8.1.a** Wetting Seamen (1979) reported that when the surface energy is low and/or the kinetic energy is high, disintegration of the droplets may occur; i.e the droplet will 'splash'. In all other cases it is suggested that the drop will distort beyond a spherical cap, recoil and in some cases rebound. Wetting of the leaf surface will occur when the kinetic energy of the droplet is insufficient to build up enough stored energy in the spreading droplet to enable it to recoil. High speed photography shows that the whole impaction process takes between 0.2-1.0 msec (Wirth *et al.*, 1991).

The tendency of the herbicide to wet a given surface may be expressed in terms of the wetting coefficient W, as follows:

$$W = \ddot{y}_{sa} - \ddot{y}_{sl} - \ddot{y}_{la}$$

Where $\ddot{y}sa$, $\ddot{y}sl$, $\ddot{y}la$ are respectively the surface-air surface tension, the surface-liquid interfacial tension and the liquid-air dynamic surface tension. Wetting of the leaf surface occurs when W is positive which occurs when:

$$\ddot{y}_{sa} - \ddot{y}_{sl} \geq \ddot{y}_{la}$$

The incorporation of a surfactant in the herbicide formulation will reduce the values of $\ddot{y}_{sl}$ and $\ddot{y}_{la}$, thus, increasing the tendency of the drop to wet the surface.

**2.8.1.b** Contact Angle Wetting is frequently expressed in terms of the contact angle (Cø) that a drop of liquid makes with a solid (Furmidge 1959 and 1962; Holly 1976; Boize *et al.*, 1976; Verity *et al.*, 1981; Reichard 1988 and De Ruiter *et al.*, 1990). This angle, measured with the droplet on a solid surface (eg a leaf), can be used to resolve vector forces in the horizontal and vertical directions. Hence the Cø may be used to balance the horizontal surface tension forces as follows:

$$\ddot{y}_{sa} - \ddot{y}_{sl} = \ddot{y}_{la} \cos\emptyset$$

Where $\emptyset$ is the angle of contact, and the other symbols are as indicated previously. By substituting this equation into the wetting coefficient equation, W now becomes:

$$W = \ddot{y}_{la} (\cos\emptyset - 1)$$

This equation clearly demonstrates that the lower the Cø and the lower the dynamic surface tension of the liquid, the solid surface becomes more readily wet by the liquid. Unfortunately, one can not make the blanket statement that a reduction in both Cø and the dynamic surface tension will enhance retention. For instance Blackman *et al.*, (1958) discovered that although a reduction in surface tension enhanced the volume of spray retained by species that were difficult to wet (eg pea and barley), it actually reduced retention on the more easily wetted sunflower and *Brassica* species.

## 2.9. Plant and environmental factors which influence spray retention.

The aim of this section is to consider how the components of the leaf surface (eg waxes) influence spray retention. Following this, consideration will be given to how the gross morphology of the leaf and the environment effect spray retention

### 2.9.1. Components of the leaf surface

The wettability of leaf surfaces is associated with the nature of the plant cuticle, with a number of reports (Challen 1959; Taylor *et al.*, 1981; De Ruiter *et al.*, 1990) regarding the composition of the cuticular surface as the most important plant characteristic determining retention, whilst leaf orientation and shape are considered to be of less importance.

The plant cuticle consists of the following components: surface wax (epicuticular), subsurface wax associated with cutin and the cutin itself (see Figure 2.9). The most important function of the cuticle is to waterproof the plant surface (Holloway, 1969). This water-repellent adaptation prevents the leaf surface from becoming saturated with water ensuring that cuticular transpiration is not hindered. Water-repellency, however, affects the deposition, distribution and retention of chemicals applied to foliage as aqueous solutions or formulations (Challen 1959; Baker and Bukovac 1971; Bukovac *et al.*, 1990).

According to Hull *et al.*, (1982) the leaf epicuticular wax represents the most important component of the cuticle as far as foliar retention of herbicidal sprays is concerned. As such, it is this component of the cuticle which will be focused on.

**2.9.1.a** **Chemical composition** The chemical composition of the epicuticular wax varies with the plant species and variations within a single genus have been shown to occur (Thomas and Barber, 1974). Epicuticular waxes contain a mixture of classes of aliphatic compounds, (Eglington and Hamilton, 1967; cited in Hull *et al.*, 1982) and it is the difference in both the nature and number of these classes that produces distinct wax compounds. Classes with odd numbers of carbon atoms are commonly alkanes and secondary alcohols, less commonly ketones and ß-diketones and infrequently ketols, alkenes, 2-methyl and 3-methyl branched alkanes. Classes with even numbers are commonly esters, primary alcohols, and fatty acids, less commonly aldehydes, a-w-diols and w-hydroxy acids and infrequently unsaturated fatty acids. Some waxes also contain large amounts of triterpenoids such as ursolic acid and oleanolic acids. The overall chain length of the homologues is usually $C_{20}$-$C_{35}$, but chain lengths may be as short as $C_{10}$ (w-hydroxy acids) and as long as $C_{64}$ (esters).

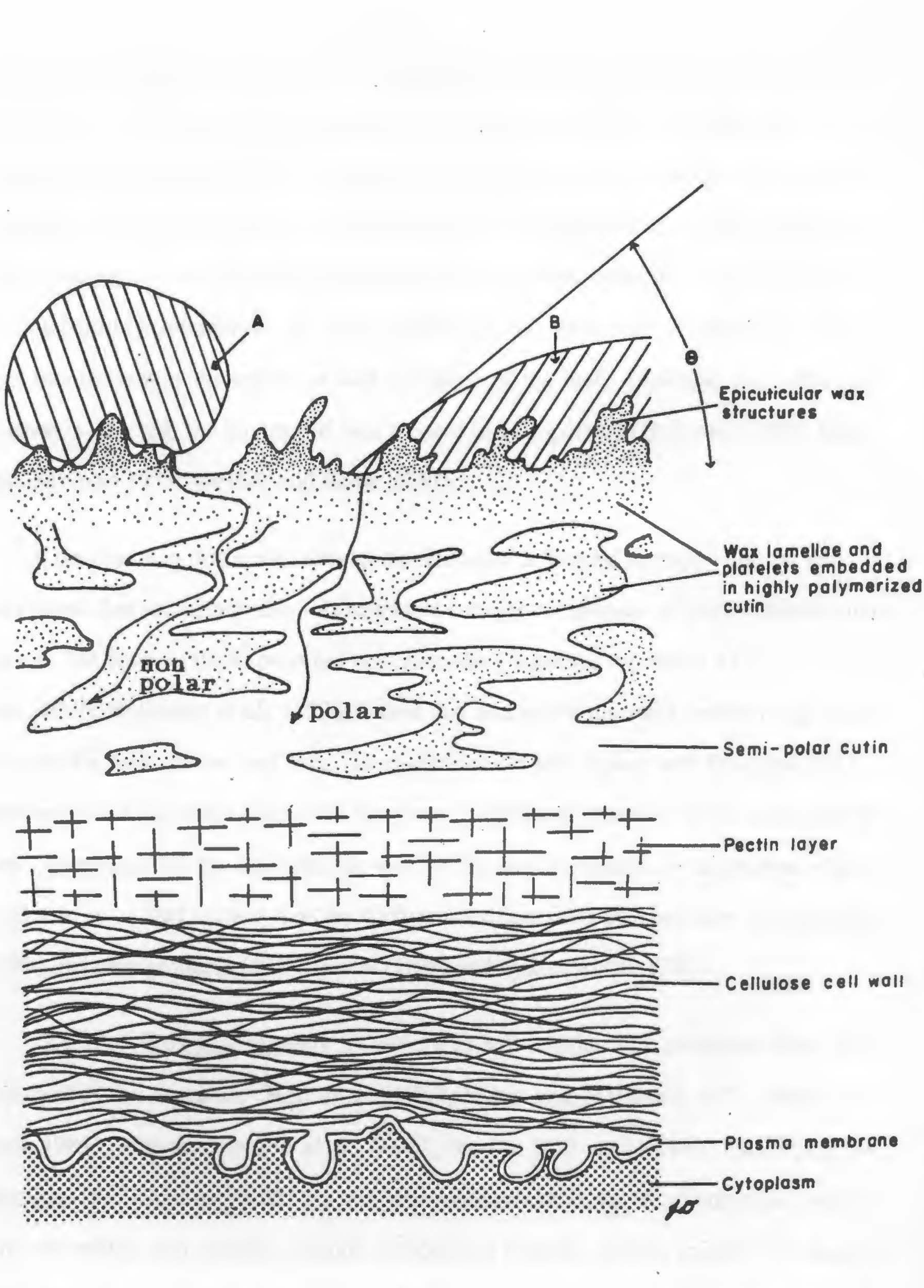



Figure 2.9: Diagrammatic representation of the leaf cuticle as seen in cross section. Surface wax structures prevent a spray droplet without surfactant (A) from contacting the cuticle proper. A droplet with surfactant (B) has a reduced contact angle (Ø), enabling it to effectively contact the leaf surface. Relative thickness of the cell wall and the various cuticular components depicted can vary markedly among different plant species, and with extent of foliar developmet. (From Hull *et al.*, 1982)

The chemical composition of the epicuticular waxes has been shown to alter wettabilty. Holloway (1969) found that those waxes containing large amounts of alkanes are least wettable, but waxes consisting mostly of esters, ketones and secondary alcohols are almost as hydrophobic. On the other hand waxes consisting of large amounts of W-diols and primary alcohols are most wettable. Just as important in determining wettability of leaf surfaces as the chemical composition of the epicuticular wax is the extent of wax coverage of the leaf. Provided the entire leaf surface is covered, the amount of wax present is unimportant (Holloway 1969; Baker and Bukovac 1971; Stevens and Baker 1987).

Of the plants in this study, data in the literature is limited to poppy and fat hen. It was found that secondary alcohols represent a major component of poppy epicuticular waxes. Holloway (1969) reported that secondary alcohols represent 41% of the leaf wax, while Holloway *et al.*, (1976) found that secondary alcohols; namely $C_{29}$-10-ol account for 66% of the leaf wax. In regards to fat hen, Baker and Bukovac (1971) reported that long-chain aldehydes represent a significant fraction of the waxes of fat hen. Investigating the epicuticular wax of fat hen in respect to bentazone action Taylor *et al.*, (1981) found that the surface wax consisted of a mixture of aldehydes (30%), primary alcohols (45%), esters (18%) and hydrocarbons (7%).

**2.9.1.b** Wax morphology A variety of epicuticular wax structures have been reported in the literature (Hall *et al.*, 1964, Baker and Holloway 1971, Baker and Hunt 1981), with Amelunxen *et al.*, (1947, cited in Hull *et al.*, 1982) classifying the structures into six basic types: (1) wax granules (spherical, short cylindrical or warty), (2) wax rodlets and threads (straight rodlets and hooked, curled, spiralled or looped threads), (3) wax plates and scales, (4) wax layers and crusts (smooth, warty or layered with rodlike or papillose appendages), (5) aggregate wax coatings (granular, rodlets or filaments) and (6) liquid and viscous wax coatings (drops or soft flat cakes).

The nature of the projections apparently makes little difference in the wettabilty of a leaf surface. Rentschler (1971, cited in Hull *et al.*, 1982) examined a number of plant species and concluded that no relationship existed between the type of wax structures and wettability. But, it was noted that the structures are readily cast off in older leaves, enhancing their wettability, as wax synthesis is greatly reduced once the leaf is fully expanded.

**2.9.1.c** **<u>Factors influencing wax formation</u>** The nature of the epicuticular waxes can change in response to environmental factors. For example, Baker (1974) showed that increased light intensity, decreased humidity and decreased temperatures resulted in larger deposits of wax. Plant surfaces can also be subject to weathering which may effect their wettability. Damage may be caused by the rubbing of leaves on the same or neighbouring plants, by rain or by the scarifying effect of wind-borne sand. Thus, environmental factors modify the nature of leaf surfaces and consequently have the potential to change leaf wettabilty and responses to post emergent herbicides.

The biosynthesis of epicuticular wax and its deposition onto plant surfaces can also be influenced by other herbicides. Dewey *et al.*, 1956 (cited in Leavitt *et al.*, 1978) first reported that trichloroacetic acid inhibited epicuticular wax deposition on peas and *Brassica* crops. The trichloroacetic acid treated plants had glossy foliage that showed increased susceptibility to foliar applied herbicides.

In 1966 Gentner reported that the herbicide EPTC (S-ethyl dipropyl thiocarbamate) decreased epicuticular wax deposition which resulted in increased levels of spray retention on cabbage (*Brassica oleracea* L.). The effect of EPTC on reducing epicuticular waxes was not immediately apparent, but became evident a short time after the spray was applied. Leaves expanded at the time of spraying were unaffected and contained the normal quantities of wax. The reduction in epicuticular wax occurred only on unexpanded leaves in the bud at the time of spray application. Flore and Bukovac (1974), in addition to confirming the results of Gentner (1966) showed that epicuticular wax deposition was inhibited on those leaves present at spraying which were not fully expanded. These findings were supported some years later by Kolattukudy (1980) who reported that wax is deposited only during leaf expansion.

As with poppies, weed control practices in the sugar beet family involve sequential herbicide applications with the herbicide ethofumesate. This herbicide has been reported to reduce the weight of epicuticular waxes on the surface of cabbage and sugar beet leaves (Leavitt *et al.*, 1978; Duncan *et al.*, 1981 and Duncan *et al.*, 1982). Inhibition by ethofumesate of deposition of epicuticular waxes occurred only on developing leaves. The reports indicated that ethofumesate increased the overall deposition of long chain esters and decreased alkane ($C_{29}$) and sec-ketone ($C_{29}$) components. The mechanism of action was explained by the possible inhibition of fatty acid elongation in the elongation-decarboxylation pathway of epicuticular wax synthesis. Duncan *et al.*, (1982) reporting the decrease in epicuticular wax caused greater retention of foliar-applied herbicide by those leaves in the early stage of development.

Similar reductions of epicuticular wax deposition on young expanding leaves was reported by Still *et al.*, (1970), when pea plants were treated with the thiocarbamate, diallate [S-(2,3-dichloroallyl)diisopropylthiocarbamate]. It was found that diallate inhibited the production of primary alcohols to a greater extent than other wax components (such as hydrocarbons, esters, secondary alcohols and fatty acids). It was not clear why the primary alcohols were preferentially affected and Still *et al.*, (1970) could not elucidate the mechanism by which diallate inhibited these compounds.

From this discussion it is clear that the production of epicuticular waxes and their deposition onto plant surfaces can be altered by herbicide applications. It is possible that the current use of foliar applied ethofumesate in poppy crops is increasing the ability of subsequent herbicides (eg diquat) to wet the leaves of weeds. Perhaps the secondary alcohols of poppy leaves are not inhibited by ethofumesate, whilst the deposition of primary alcohols on young leaves of fat hen are. It is only through a detailed analysis of the epicuticular wax components of those plants under study that such questions can be answered. Such studies have not previously been reported in the literature.

### 2.9.2. Leaf age and morphology

The wettability of leaf surfaces is closely related to leaf structure. This was demonstrated as early as 1927 when Aslander, (cited in Holly, 1976) showed that sulphuric acid damages the cotyledons of red clover but the leaves are protected against the herbicide by their coating of hairs which prevents intimate contact with the actual surface. Similarly, Furmidge (1962) observed that the dense covering of hairs on the surface of young apple leaves prevents drops from wetting the leaf surface; air is trapped below the drop, resulting in large contact angles. The opposite effect can occur, in that a hairy leaf is readily wetted by the herbicide spray. The hairs can form a weak irregular mesh which may be easily wetted, therefore, increasing herbicide contact with the leaf surface.

The age of the plant is influential in determining the orientation of the leaves. Leaves of monocotyledons are nearly vertical when they are seedlings and become horizontal as they elongate and expand, with the tips bending towards the vertical as the leaves continue to expand and mature. Seedlings of dicotyledons tend to have leaves that are nearly vertical when they first emerge, and quickly become horizontal. The orientation of leaves may then range from horizontal to varying angles, depending on the species and the age, and whether or not the plant is isolated or crowded by other plants. Therefore, a variety of leaf angles may be present in any given field. Since retention is partially a function of leaf angle, (there being a greater likelihood of a falling drop being reflected from a leaf if the angle of contact between the drop path and the leaf surface is small) it may vary considerably, depending on the individual plant species and stage of development (Hull *et al.*, 1982).

Differences in retention, with respect to leaf age were reported by Hibbitt (1969, cited in Holly, 1976), when investigating the selectivity of asulam between wild oats (*Avena fatua* L.) and linseed (*Linum usitatissimum* L.). An increase in retention with increasing age was reported for wild oats while a decrease in retention with age was experienced with linseed. In both cases the volume of spray retained was directly related to the plan view area as obtained by vertical projection, irrespective of age. Davies *et al.*, (1967, cited in Holly, 1976) reported that of the total leaf area of pea and barley, the projected leaf areas were approximately 40% and 13% respectively. There was in each species a considerable amount of leaf overlap, but according to Davies *et al.*, (1967, cited in Holly, 1976) these figures largely reflect leaf angle differences and suggest that on interception alone, barley plants would only receive one-third of a vertical falling spray when compared with pea plants. In both reports the importance of the inclination of the target surface, in regards to herbicide retention was highlighted.

The important role epicuticular waxes play in influencing spray retention was discussed earlier, and just as leaf angle and area vary considerably with age so does the morphology and amount of epicuticular wax deposits on leaves. A survey of the literature produced a range of reports in regards to whether leaves become progressively easier or harder to wet with age. Linskens (1952, cited in Furmidge, 1962) showed that as a daffodil (*Narcissus pseudonarcissus* L.) leaf develops, the Cø of water on its surface increases to a maximum during the growth of the leaf and then decreases as the leaf ages. Likewise, Thompson (1958) considers that young leaves are more difficult to wet than older ones. These observations can be explained by the fact that the rate of wax synthesis during expansion does not compensate for the rapid increase in surface area that occurs as the leaf expands. Consequently the wax deposits present on immature leaves decrease in the course of development.

When measuring the responses of onion (*Allium cepa* L.), speedwell (*Veronica presica* Poir.), chickweed (*Stellaria media* L.) and rayless mayweed (*Matricaria *matricarioides* Less.) to post emergent applications of methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiaxolidine-3,5-dione], Verity *et al.*, (1981) found that onion plants became more tolerant to herbicide applications the older they were at the time of treatment. On the other hand, herbicide retention on weed species did not change appreciably with age. The greater tolerance to methazole experienced by older onion plants was due to a decrease in herbicide retention bought about by a progressive increase in the deposition of epicuticular waxes.

At first glance one might regard the findings of Thompson (1958) (ie young leaves more difficult to wet than older leaves) and Verity *et al.*, (1981) (ie old leaves more difficult to wet than younger leaves) to be in conflict. However, what these reports have demonstrated is that as leaves age there will inevitably be differences in the ratio of the quantity of leaf epicuticular wax to leaf area. By determining how epicuticular wax deposits are effected on crop and weed leaves with age, it may be possible to take advantage of any differences for selective weed control.

## 2.10. Penetration

To be effective, herbicides must enter the plant. Some plant surfaces will absorb herbicides quickly, but other plant surfaces absorb the chemicals slowly, if at all. The chemical nature of the herbicide formulation will also influence penetration. Therefore, selective penetration of herbicides may account for differences in plant responses.

Initial leaf penetration of herbicides may take place either through the leaf surface or through the stomates. Foliar uptake has been correlated with stomatal frequency (Stevens and Baker, 1987), with Boize *et al.*, 1976 reporting that stomata are important routes for the penetration of spray drops. This preferential foliar absorption in the region of the stomata may be related to the thinner cuticle and/or reduced wax deposits which overlie the guard cells (Stevens and Baker, 1987). Most reports, however, regard direct penetration of the leaf surface by foliar applied herbicides of more importance (Klingman and Ashton, 1982). Here the herbicide must first penetrate the cuticle, which as discussed in the previous section is not homogenous in composition (see Figure 2.9).

There is a gradual transition in the polar nature of the cuticle cell wall complex from the epicuticular wax to the cellulose. The epicuticular wax is most non-polar (hydrophobic), followed by cutin, pectin and cellulose, which is in fact polar (hydrophilic), (Klingman and Ashton, 1982). Therefore, polar compounds have considerable difficulty entering the epicuticular wax, but once they pass this barrier they enter each succeeding phase more readily. In contrast, non-polar compounds readily enter the epicuticular wax but have increasing difficulty in passing into successive phases. Thus, the polar nature of the herbicide formulation will have a considerable influence on the rate of penetration. Figure 2.9 shows the hypothetical routes of entry of both polar and non-polar herbicides.

The amount of herbicide penetrating into the leaf as a proportion of that deposited upon its surface is often low (Holly, 1976). However, there is a general presumption that if the formulation is changed so as to bring the herbicide into more intimate contact with the leaf surface the penetration of the herbicide shall increase. As seen in Figure 2.9 this increased contact can be readily achieved, due to those reasons mentioned earlier (eg lower contact angle and reduced dynamic surface tension of the herbicide formulation) through the addition of surfactants. Yet, surfactants can have a differential effect on penetration between species as illustrated by Holly (1976). The addition of the surfactant 'Lissapol NX' to paraquat gave a eightfold increase in penetration by cocksfoot (*Dactylis glomerata* L.), due to a marked increase in retention but had no effect on tomato (*Lycopersicum esculentum* Miller.). This work must be interpreted with caution, as work performed by Bland and Brian (1975, cited in Seamen, 1979) on the uptake and movement of paraquat in the presence of non-ionic surfactants, found that although surfactants can enhance uptake of pesticides they may inhibit movement within the plant.

### 2.10.1. Environmental factors influencing herbicide penetration.

The environmental factors which alter foliar penetration of herbicides are very complex. Under field conditions, light, temperature and humidity interact, and quantification of the relative significance of each is difficult. Firstly, light, temperature, and humidity may exert their effect during the absorption process, or they may influence plant development prior to absorption, resulting in a decrease or in increase in foliar penetration of a given herbicidal dose (Bukovac, 1976).

**2.10.1.a Effect of environment during penetration** Sargent and Blackman (1965, cited in Bukovac, 1976) found that penetration of 2,4-D into bean and sugar beet were enhanced by light, and relatively low intensities (5,000-15,000 lx) were adequate for maximum response. In contrast, Brian (1967, cited in Bukovac, 1976) reported that there was greater penetration of diquat and paraquat into tomato leaves in darkness than in light. Although these reports appear to be conflicting they highlight that the effect of light/dark conditions on penetration will depend on the plant/herbicide combination. One must also remember that photochemical decomposition of herbicides does occur and, hence, under high light intensities the herbicide dosage may decrease with time (Bukovac, 1976).

In work reviewed by Bukovac (1976) it was reported that foliar penetration of herbicides is favoured by high relative humidities (R.H). High R.H increases the drying time of spray droplets, favours stomatal opening, enhances transport and may increase the permeability of the cuticle.

According to Hull *et al.*, (1982) temperature has a greater influence on the penetration of herbicides into leaves than light or R.H. Within biological limits penetration rates increase with increased temperatures. However, with too high a temperature, volatilization of the herbicide occurs, and if the spray dries, the volume of herbicide that remains on the leaf surface will be drastically reduced.

**2.10.1.b** <u>**Effect of environment prior to treatment**</u> The environment under which a plant develops may markedly effect the penetration of a herbicide subsequently applied to the foliage.

A number of reports, reviewed by Hull *et al.*, (1975) support the contention that cuticle development is proportional to light intensity. A possible implication of this observation is that those leaves which develop at the top of the canopy (eg under full sunlight) would present a greater barrier to the penetration of herbicides than those leaves which develop at the base of the canopy (eg in the shade).

Hull *et al.*, (1975) also investigated the influence moisture stress played in cuticular development. Surprisingly it was revealed that variations in soil moisture stress within a single species had no influence on the size or composition of the leaf cuticle. Hence, moisture stress would appear to have no effect on the penetration of foliar applied herbicides.

Predisposing plants to high (20° to 30°C) temperatures and R.H (70 to 100%) results in greater penetration, due to a decrease in epicuticular wax deposition under these conditions than when plants are predisposed to low temperatures and low R.H. (Bukovac, 1976). Although the temperature and R.H may vary within a leaf canopy, it would appear very doubtful if such variables could be manipulated for the selective penetration of herbicides.

## 2.11. <u>Herbicide Interactions</u>

Either synergistic or antagonistic, herbicide interactions are a result of both physical and chemical changes which may cause the herbicide mixture to perform differently from any single component of the mixture applied separately. Such interactions are of special concern for weed control and crop safety and have fittingly received wide attention. Eshel *et al.*, (1976), Duncan *et al.*, (1982), Andrews (1990) and undoubtedly many others have investigated these interactions and their ramifications. Yet the mechanisms by which herbicides interact in mixtures are complicated and usually unknown (Akobundu *et al.*, 1976).

Often compounds in the mixture may effect each other by interfering with the course of penetration, translocation, or metabolism exhibited by any single compound. They may also effect herbicidal activity at the site of action within the plant cells by disrupting physiological and biochemical processes. These interferences and disruptions are frequently the cause of herbicide interactions. However, Eshel *et al.*, (1976), working with a mixture of ethofumesate and desmedipham, found that additional complex interactions may take place in mixtures of herbicides formulated with organic solvents and adjuvants. Without exhibiting a phytotoxic effect themselves, the formulants of ethofumesate significantly increased the activity of desmediphan. It appears likely that a similar effect takes place upon the addition of diclofop-methyl to diquat. Diclofop-methyl, like ethofumesate is formulated as an emulsifiable concentrate, and following the work of Eshel *et al.*, (1976) it seems that it is not the active constituent, but rather the organic solvents and adjuvants of the formulated product causing the synergistic reaction. This raises the question concerning the extent to which these formulants may be considered as inert ingredients.

## 2.12. Conclusion

Variations in plant response to foliar-applied herbicides may be explained by one or a combination of several factors (Holly, 1976). It was recognised in early studies (Blackman *et al.*, 1958; Furmidge, 1962) that differences in spray retention on different plant species, or even different varieties of the same species was one of these factors. When spray drops impact on the leaf surface they flatten, recoil and subsequently are retained or reflected. The outcome depends on the physical properties of the spray solution and the physical and chemical nature of the leaf surface (De Ruiter *et al.*, 1990; Grayson *et al.*, 1991). The influence of the physical properties of spray has been investigated by several workers (Singh *et al.*, 1984;

Wirth *et al.*, 1991), and although parameters such as drop size and velocity are regarded as significant there is general agreement amongst researchers that the dynamic surface tension of the spray liquid provides the best correlation with retention. It has also been widely recognised that contact angles give relevant information on the wettabilities, and hence potential for spray retention, of leaf surfaces (Furmidge, 1962; Boize *et al.*, 1976; Singh *et al.*, 1984). In regards to the physical and chemical nature of leaf surfaces one can discriminate between plant canopy and shape, leaf orientation, and macro-roughness (eg leaf hairs). However, according to Hull *et al.*, (1982) and De Ruiter *et al.*, (1990) the composition and quantity of leaf waxes are the main retention deciding factors. Holloway (1969) studied the chemical composition of isolated individual leaf waxes in relation to their wettability and found that variations in the type and number of chemical groupings, exposed on the surface greatly modified spray retention. It should also be emphasized that leaf surface characterisitics, physical and chemical, vary greatly between species, and for any one species they can vary with plant age (Verity *et al.*, 1981).

In examining the mechanisms of herbicide selectivity, regardless of the crop, each of the above mentioned parameters must be considered. However, in the case of poppies further attention must be directed towards the effects of herbicide interactions. Physical and chemical changes may cause the diquat/diclofop-methyl mixture to interact, thus, enabling the herbicide formulation to perform differently from either component when applied separately. Equal consideration must also be given to the possibility of an interaction between the first and second spray applications.

# 3. Materials and Methods

# 3 MATERIALS AND METHODS

## 3.1 Plant species and growing conditions

The following species were used *Capsella bursa-pastoris, Chenopodium album, Cirsium vulgare, Fumaria muralis, Raphanus raphanistrum, Rumex crispus, Papaver rhoeas,* and *Papaver somniferum* (cv. C023-27-5). The plants were grown in plastic pots from seed in a glasshouse situated at the University of Tasmania, Hobart (see Plate 1.0). Seed of *P. somniferum* was provided by Tasmanian Alkaloids PTY. LTD.

Temperatures in the glasshouse were maintained between 15/25 C (night-day), while relative humidity varied between 50% and 70%.

The 15cm diameter pots were filled with a mixture of sand and Tasmanian peat moss (1:1). A slow release fertiliser (osmocote), dolomite, and lime were added. The seeds of *P. somniferum* were treated with the fungicide Mistisan, while all weed seeds were treated with Thiram, a dithiocarbamate used to control any fungal contamination. When sown the seeds were watered in with tap water, and after emergence received light watering daily.

After emergence the seedlings were thinned to six per pot.

Field trials were conducted at both east and north-west Tasmania on commercial crops of poppies. Each site was chosen because it was heavily infested with *C.album* (fat hen) and *F.muralis* (fumitory).

## 3.2 Spray Application

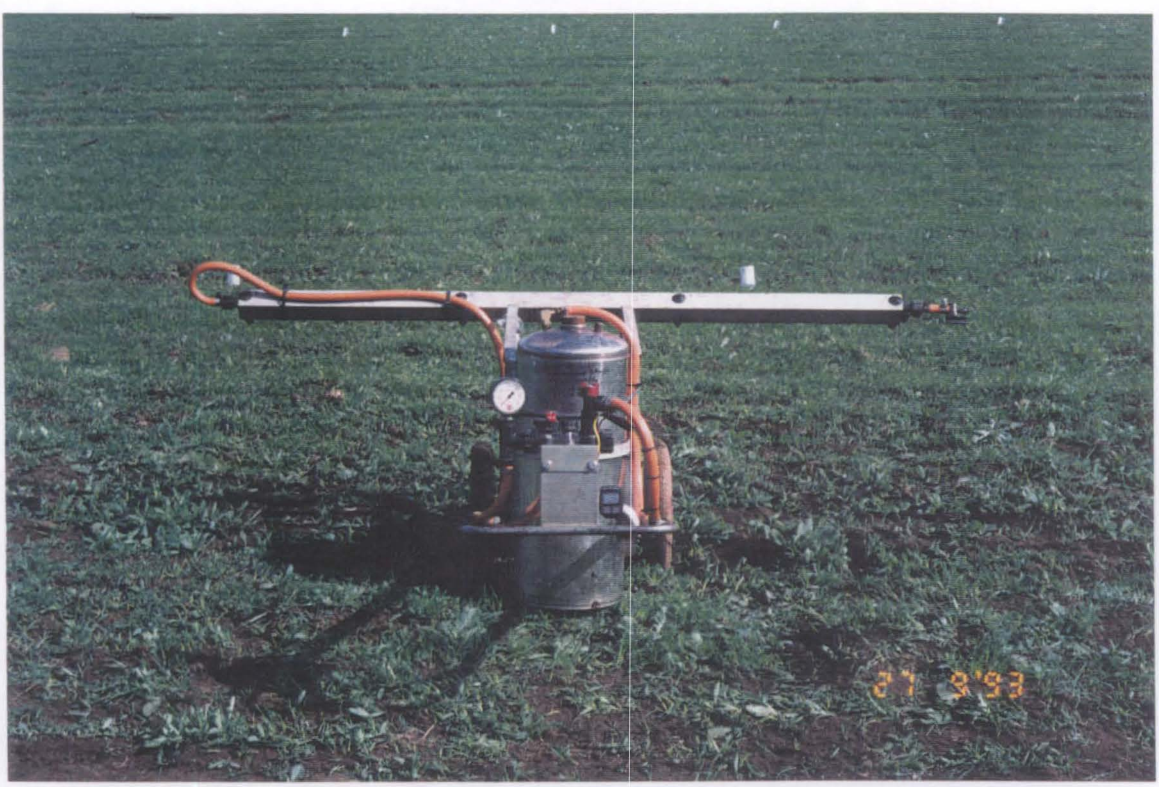
For the determination of leaf contact angles a one microliter droplet was placed on the leaf surface using a Hamilton #7001 syringe (see Experiment 1, Plate 3.0). For field measurements the spray solutions were applied at 0·8 bar using a field plot sprayer fitted with four, size 14 flat spray nozzles (see Plate 2.0). The nozzles were fitted on the spray boom at intervals of 50 cm, while the distance between the nozzles and the ground was also 50 cm. The volume mean diameter of spray droplets was estimated at 300 µm, while the application rate was 200 l/ha. Deionised water was used in preparation of all laboratory applied spray solutions, whilst in the field, rain water which is the conventional spray carrier, was used. Plants were sprayed when they had reached the 4-6 leaf stage, which corresponded to a period of 5-6 weeks from the date of sowing. When required the asulam/ethofumesate treatment was applied, using a 1 L hand pump operated Garden Sprayer, within four to six days prior to any investigation or subsequent treatment.

## 3.3 Statistical Analysis of Results

Experiments were conducted as randomised complete block designs, and unless otherwise stated, each treatment was replicated three times. Statistical analysis were performed using SAS and Ministat.

**Plate 1.0**
**Glasshouse plants 6 weeks after sowing.**

**Plate 2.0**
**Field plot sprayer.**



# 4. Experiment 1

# 4 EXPERIMENT 1 Contact Angles of Herbicide Solutions as Affected by Plant Species and Surfactants

## 4.1. Introduction

The amount of herbicide retained by a given plant species is of primary importance when one is considering the selective phytoxicity of a herbicide solution. The measurement of contact angles has been one of the most widely used methods of predicting herbicide retention, it not only characterises the wettability of the leaves but it also determines the form of the spray droplets on the leaf surface and hence their behaviour.

The retention of spray solutions on solid surfaces has been discussed by Furmidge, (1962) who points out that where the values of the contact angle are low (<70°) the retention of spray is greater than where the contact angle is very high (>90°). The differential wetting of crop and weed species by a spray solution, due to variations in contact angle, is often utilised for selective post emergent weed control. The inclusion of a surfactant to the spray solution will, depending on the concentration, alter the differences that previously existed in surface wetting between plant species, which will in turn modify the efficacy of the herbicide solution.

With regard to poppy production, combinations of several surfactants have been added to herbicides to determine their effect on efficacy, yet no work has been done on the quantitative determination of contact angle. The following experiment was conducted to examine:

- the nature of diclofop-methyl and its effects, when formulated with diquat, on the contact angle of poppy and a number of weed species frequently encountered in poppy crops.

- the contact angle for diclofop-methyl and two surfactants at various concentrations of the latter.

- to devise a simple technique which could be used to assess surfactant suitability for application in combination with diquat. If a surfactant appears adequate at this level it could be evaluated further in a field trial. The screening of surfactants at an early stage would prove more efficient than the current technique of field testing each surfactant of interest.

Finally, through this experiment it was the intention to gain an appreciation of the wettability of poppy plants and the weeds commonly found in poppy crops.

## 4.2. Materials and Methods

In all treatments, except that of the control, the concentration of diquat was maintained at the rate of 1 L product/ha (0.5%). Diclofop-methyl, Newkalgen® (a yet to be released cationic surfactant) and Agral®, were added to the herbicide solution at concentrations ranging from 0.001% to 10% (v/v). Contact angle measurements were conducted on untreated plants, then on plants treated with asulam and ethofumesate (see Experiment 3).

Upon application of the treatment mixtures the relative wettability of the selected weed species and poppy plants was examined through contact angle measurements. A Pradavit n24 slide projector was used to project the image of a droplet, located on a horizontal leaf surface, onto white paper. A projector slide was adjusted so as a small glass slide (approximately 10 x 25 mm) could be placed on it in a horizontal position (see Plate 3.0 and 3.1). Small leaf sections (approximately 5 x 15 mm) were taken from the first fully expanded leaf of each plant. Each section was placed onto a slide, adaxial surface uppermost, by means of double sided adhesive tape. When handling the leaves, care was taken to avoid damage to the area selected for measurements, and where possible major veins were excluded. A Hamilton #7001 syringe (see Plate 3.0) was used to deliver a one microliter droplet of the herbicide solution onto the leaf section. The projected image of the droplet was traced onto white paper (see Plate 4.0). From this traced image contact angles were measured from both sides of the drop, and the average of readings was recorded. A mean equilibrium contact angle was determined from six measurements on each of three independently prepared leaf surfaces. All results and statistical analysis appear in appendix 1.

**Plate 3.0**

**Projector and syringe.**

Plate 3.1
Leaf section on glass slide.

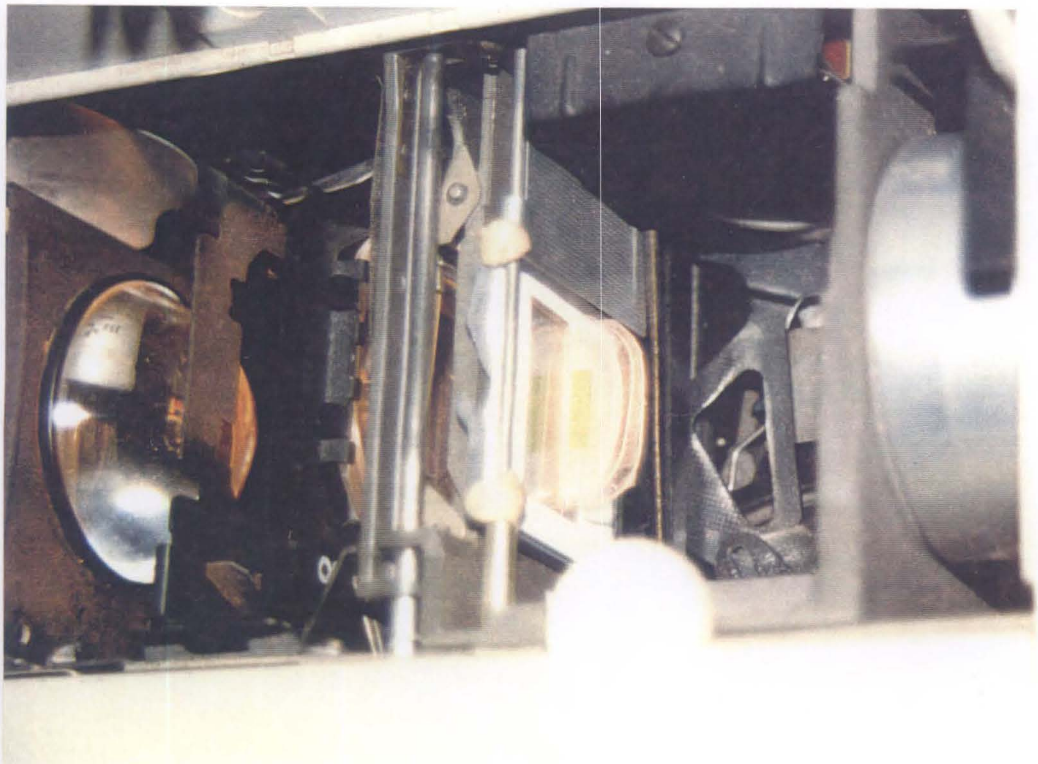
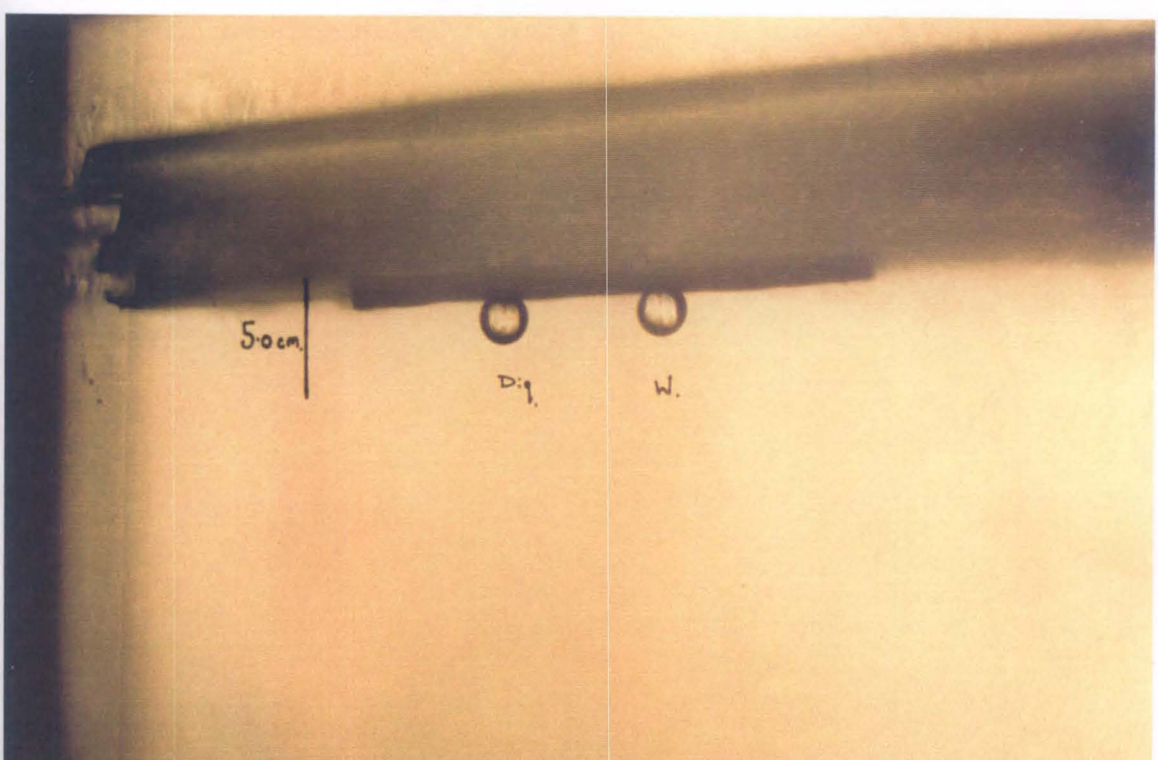


Plate 4.0
Projected image on poppy leaf.
(Diq=diquat:W=water)

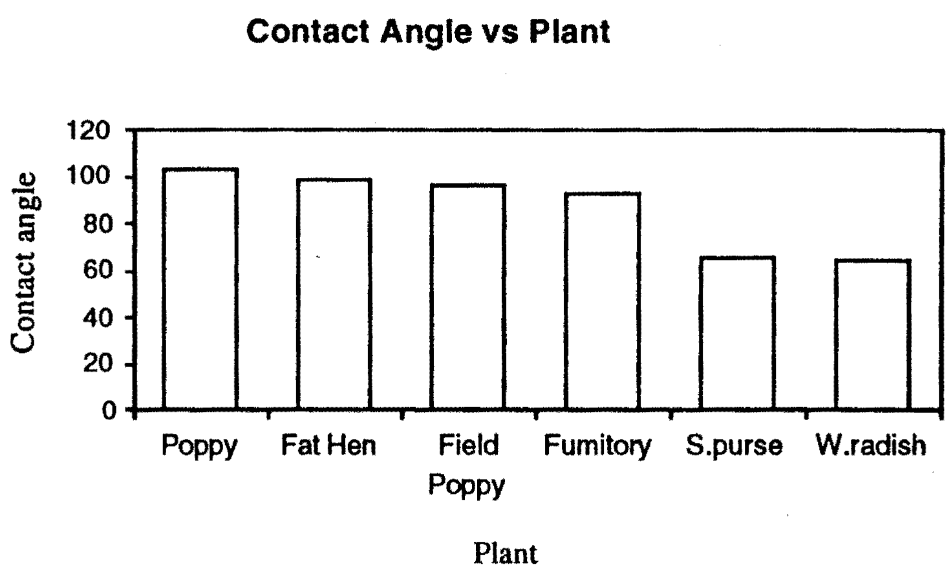


## 4.3. Results and Discussion

### 4.3.1. Effect of plant species

By examining the contact angle made by each plant species with a range of given herbicide/surfactant solutions, it is possible to determine if the selectivity experienced in the field is due to a difference in wettability of leaf surfaces. Figure 4.1, (Appendix 1) summarises the results for this experiment..

**Figure 4.1.**




Contact angles for the herbicide solutions varied significantly between species, ranging from 103° for poppy to 64° for wild radish. Although reference to all examined species could not be found in the literature, the results obtained for poppy and fat hen are in accordance with those determined by Holloway (1969), Seaman (1979) and Taylor *et al.*, (1981). As seen in Figure 4.1, the contact angle of poppy was significantly different to all weed species examined. The values for field poppy and fat hen were similar, likewise there was no significant difference between fumitory and field poppy. There was a pronounced difference between the contact angles on shepherds purse and wild radish compared with all other species examined, however, there was no significant difference between these two species.

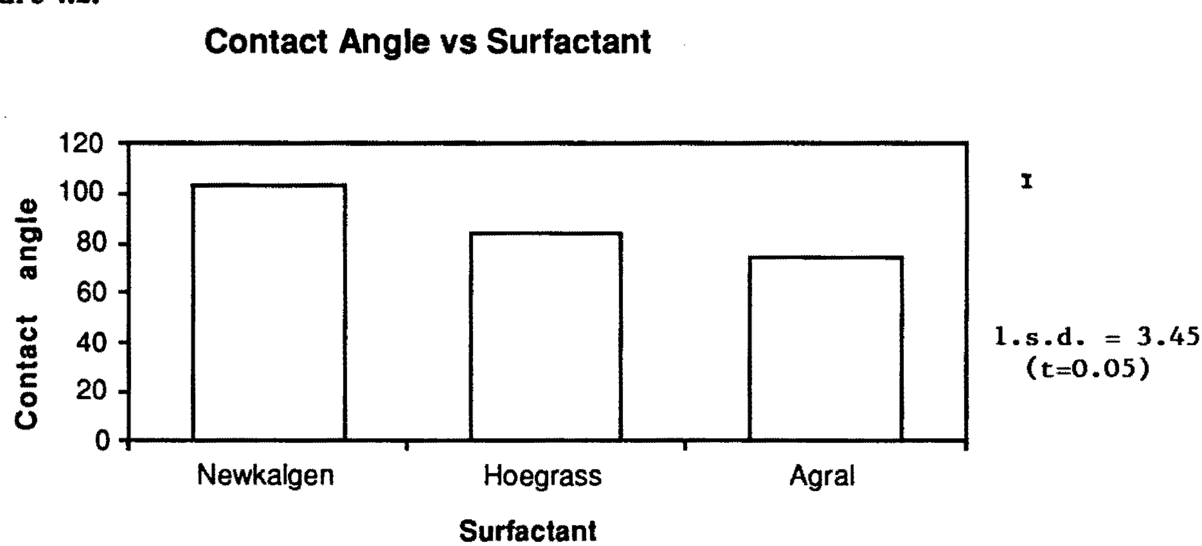
As mentioned in the introduction an angle less than 70° is characteristic of a readily wetted leaf surface, whilst angles greater than 90° are indicative of harder to wet leaf surfaces. From Figure 4.1 it is apparent that poppy, fat hen, field poppy and fumitory have angles greater than 90°, and although significant differences exist between species, these plants can be collectively classed as 'hard' to wet plants. Wild radish and shepherds purse formed angles much less than 90°, and can be regarded as 'easy' to wet plants.

Contact angles were also determined for spear thistle and curled dock. As seen in Appendix 1 both these plants were easily wetted with diquat alone. Incorporating a surfactant with diquat for use on these species would in fact reduce the quantity of spray retained on leaf surfaces due to an increase in surface run-off. For this reason, no further measurements were made using these species.

### 4.3.2. <u>Effect of surfactant</u>

By examining the ability of a surfactant, when incorporated with diquat solutions, to lower contact angles, this experiment predicts the potential of that surfactant for further use in herbicide trials. Figure 4.2, (Appendix 1) illustrates the results.

**Figure 4.2.**



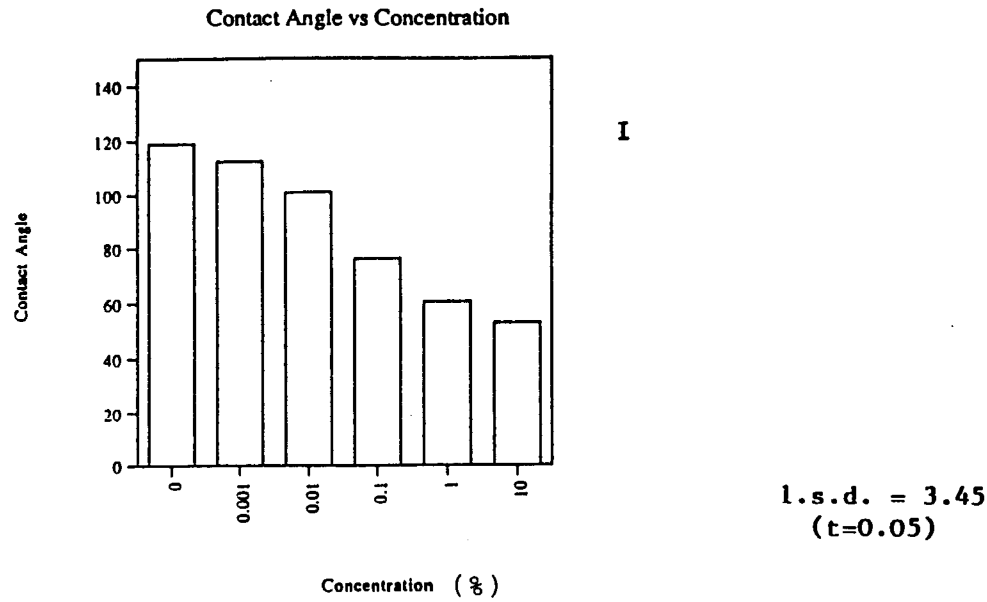
It should be noted that, without the addition of a surfactant, the contact angle made by diquat was 119°; this is an average over all leaf surfaces, both 'hard' and 'easy' to wet. Each surfactant decreased this angle significantly; ($p=0.0001$), and as demonstrated in Figure 4.2 there was a pronounced difference in the ability of each surfactant to lower this angle. Agral® was the most effective, reducing the angle to 74°, diclofop-methyl reduced the angle to 84°, whilst Newkalgen® had the least effect reducing the angle to 103°.

From the data presented, it is apparent that diclofop-methyl is acting as a surfactant, in addition to its normal herbicidal activity, when incorporated with diquat. Without this adjunct, diquat would not wet the leaves of many of the weed species (eg fumitory, fat hen and field poppy) found in poppy crops. Instead, the herbicide would splash or rebound from the leaf surface, a concept illustrated by Seamen (1979), and discussed in the literature review. From the data, it appears that Agral® would be the strongest surfactant for use with diquat. However, the ability of this surfactant to reduce contact angles, and in turn increase wetting of 'hard' to wet plants, may be so great as to decrease the margin of selectivity between poppy and weed species. In fact, such a reduction in selectivity has been observed by Matthews (pers. comm. 1993), whereby the addition of Agral® to diquat, even at low concentrations, resulted in desiccation of both crop and weeds. Therefore, it would appear that a weaker surfactant, that does not have a dramatic effect on contact angles, (eg Newkalgen® or diclofop-methyl) may be most suited for selective weed control in poppies.

### 4.3.3. Effect of surfactant concentration

With an increase in surfactant concentration, from 0 % (v/v) to 10 % (v/v), there was a corresponding decrease in contact angle. Figure 4.3, (Appendix 1) illustrates the results obtained.

**Figure 4.3.**




Over the range of surfactant concentrations tested, each significantly decreased the angle of contact the herbicide/surfactant solution made with the leaf surface, there being a pronounced difference between the lowest and highest concentrations. As seen in Figure 4.3, the increase in surfactant concentration from 0.01 % (v/v) to 0.1% (v/v) decreased the contact angle sharply. This is consistent with the finding that the maximum potential of a surfactant to lower the interfacial tension of a solution, and hence the contact angle, is reached in the region of 0.01% to 0.1% (Parr and Norman, 1965 and Foden 1972). As discussed in reviewing the literature, this region or critical micelle concentration (c.m.c), is associated with abrupt changes in many characteristic properties of the surfactant (eg changes in osmotic pressure). Although after the c.m.c was reached there was a significant decrease in interfacial tension, it would be of no practical advantage to increase the concentration of those surfactants examined beyond this range (ie greater than 1%).

As seen in Appendix 1, the experimental error associated with contact angle measurements, was small. This validates the technique developed as a reliable, repeatable procedure for evaluation of contact angles. Therefore, by measuring contact angles on common weed species and poppy plants it should be possible to evaluate a new surfactant.

# 5. Experiment 2

# 5 EXPERIMENT 2 Dynamic Surface Tension of Herbicide Solutions Affected by Surfactants

## 5.1. Introduction

Contact angles measured in the previous experiment are predominantly a function of equilibrium surface tension. However, according to De Ruiter *et al.*, (1990), Grayson *et al.*, (1991) and Wirth *et al.*, (1991) the wettability of a surface should be related to the value of the contact angle of a drop at the *moment* of impact. Such a measurement of contact angle is clearly inaccessible. However, by examining the dynamic surface tension (DST) of herbicide solutions this problem can be overcome. The above workers have demonstrated a strong correlation between retention of spray solutions and DST, that is for a low DST there is a greater probability of droplet retention.

In this experiment, the ability of the earlier examined surfactants to reduce the surface tension of diquat solutions (under equilibrium and dynamic conditions) have been determined. This information could be used to correlate the effect of these surfactants on herbicide retention. It will also provide an indication of the effect of measuring contact angles under equilibrium conditions, as opposed to dynamic conditions.

## 5.2. Materials and Methods

The DST of diquat and a number of diquat/surfactant combinations was measured at ICI Chemicals and Plastics (Surfactants Division) using the maximum bubble pressure method (MBPM). Details of the MBPM are given in Appendix 2. The method was checked using distilled water (DST 72·8 $mNm^{-1}$ @ 20°C). The surface tension of each treatment was measured at near equilibrium conditions (frequency = 2Hz) through to very high dynamic conditions similar to those experienced by a droplet imparting on a leaf surface (frequency = 30Hz). These measurements were performed at surfactant concentrations between 0.01% (v/v) and 0.1% (v/v), (ie in the c.m.c). As there was no replication of treatments statistical analysis was not performed on the results.

## 5.3 Results and Discussion

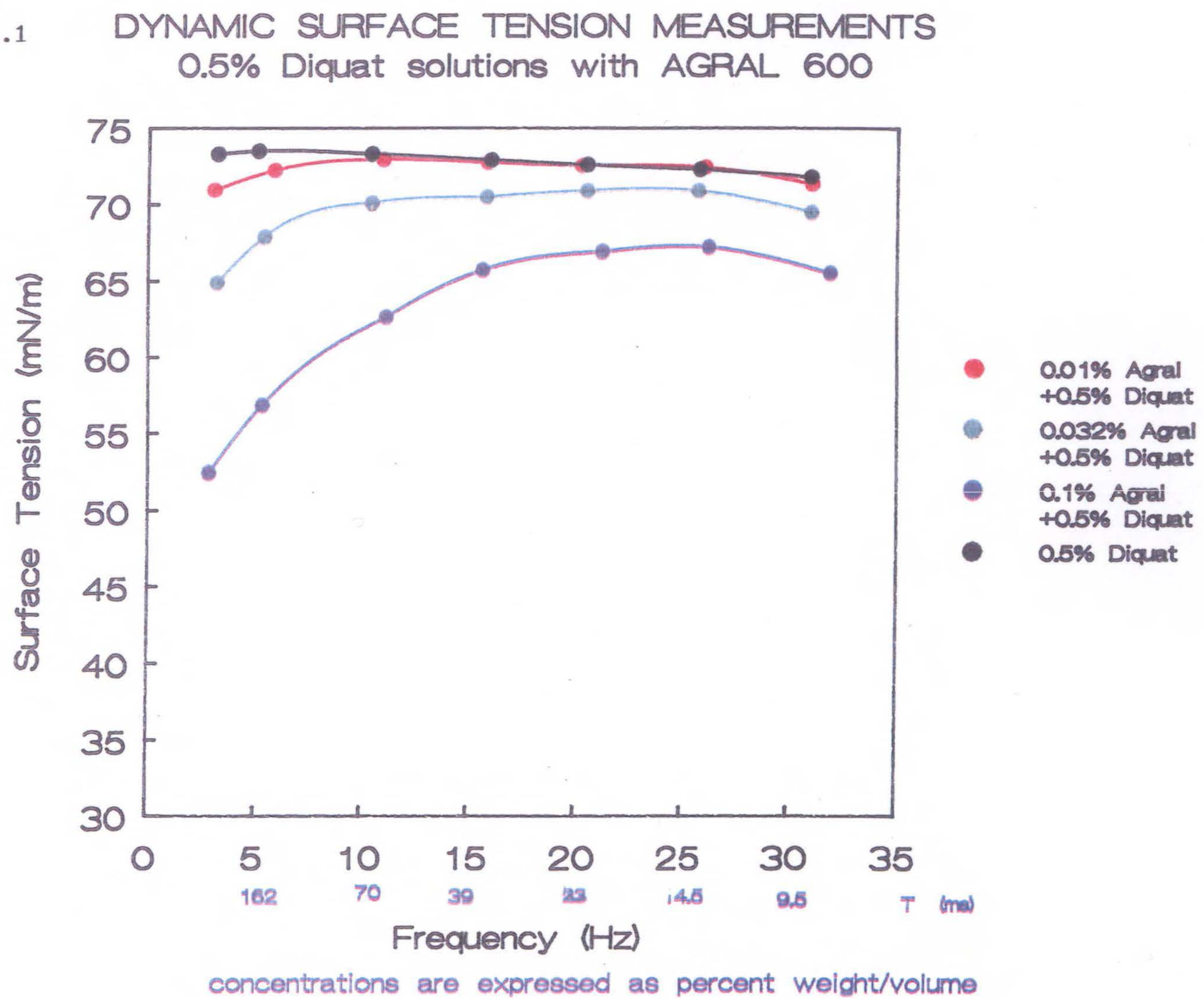
The results are presented in the form of surface tension vs bubble frequency graphs. Data for these figures are shown in Appendix 2. In general, it was observed that an increase in surfactant concentration resulted in a decrease in surface tension, this decrease being greater under equilibrium conditions.

Looking at Figures 2.1 to 2.3, the response of diquat to surfactant was most apparent with Agral® and Newkalgen® and less noticeable with diclofop-methyl. However, at a concentration of 0.1% (v/v) diclofop-methyl was observed to reduce the DST, thus supporting the claim made in Experiment 1 that this herbicide has properties characteristic of a surfactant.

Unfortunately, DST measurements were not performed beyond the surfactant concentrations observed here. In future, such measurements could be pursued over the range of concentrations examined in Experiment 1. These results could then be correlated with herbicide retention. By examining surfactants in this manner it may be possible to find a concentration of a surfactant, say Newkalgen®, that when incorporated with diquat performs similarly to diclofop-methyl at the concentration it is currently used in herbicide programs (ie 1.0% v/v).

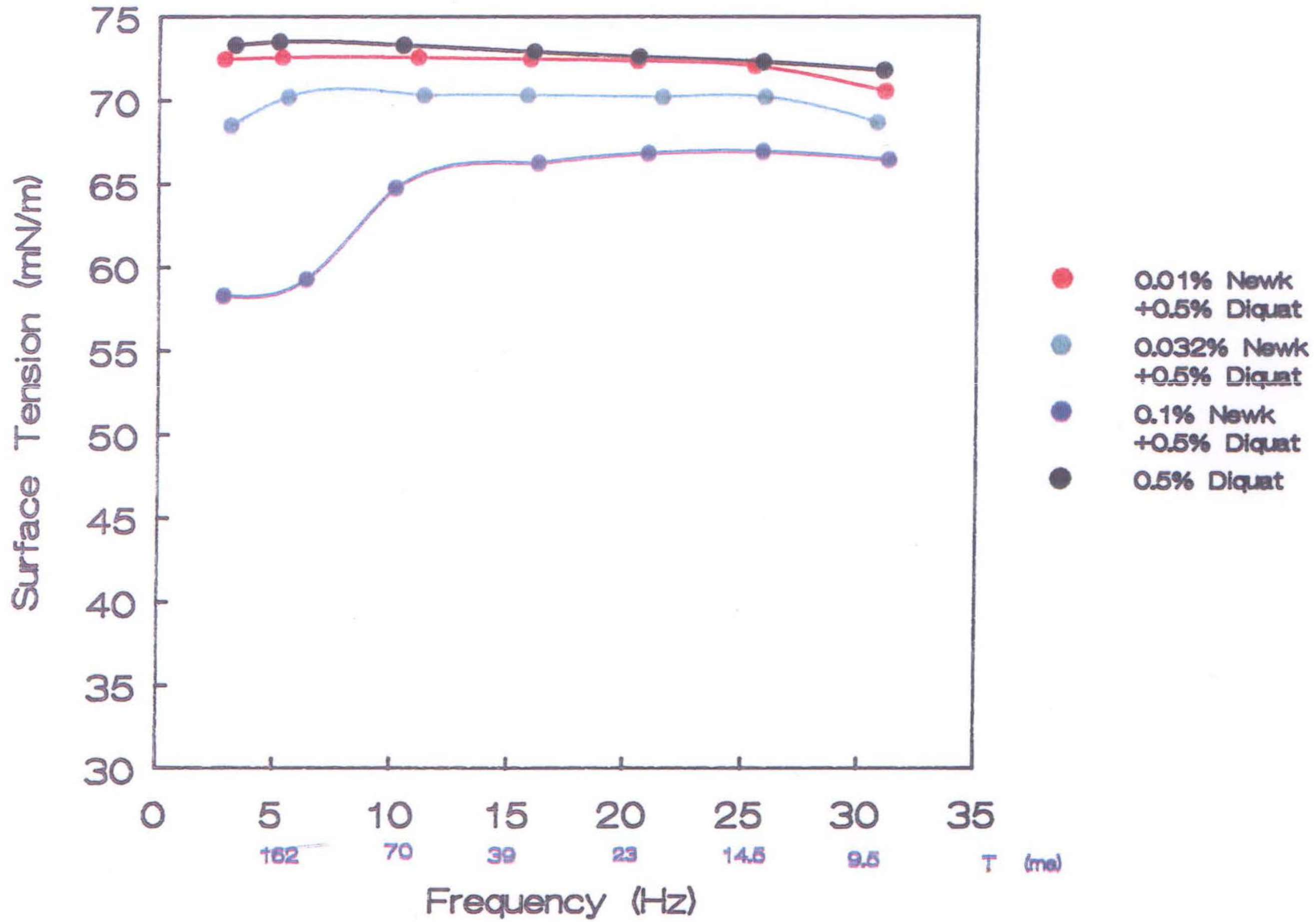
It was mentioned earlier that under equilibrium conditions surface tensions appeared to be less when compared with measurements made under dynamic conditions. With this in mind, it is important not to attempt to directly relate contact angle measurements made in the first experiment to any absolute determination of herbicide retention. Such measurements would tend to overestimate the quantity of herbicide retained at a given surfactant concentration. However, contact angle measurements are still a valid comparative measurement, suitable for comparing surfactants and wettability of plant species.

Figure 2.1



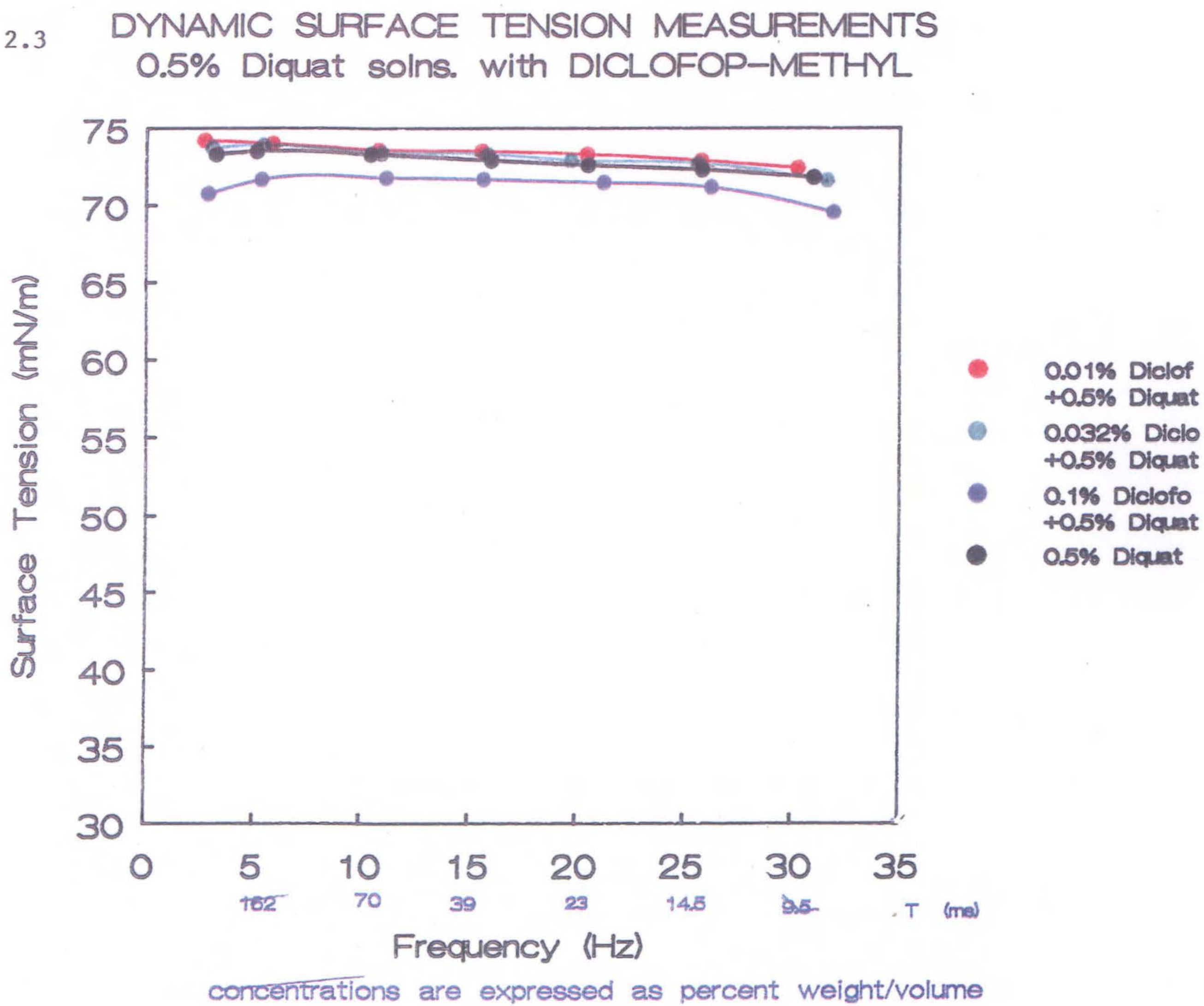
DYNAMIC SURFACE TENSION MEASUREMENTS
0.5% Diquat solutions with AGRAL 600
Surface Tension (mN/m)
75
70
65
60
55
50
45
40
35
30
0
5
10
15
20
25
30
35
162
70
39
23
14.5
9.5
T (ms)
Frequency (Hz)
concentrations are expressed as percent weight/volume
0.01% Agral +0.5% Diquat
0.032% Agral +0.5% Diquat
0.1% Agral +0.5% Diquat
0.5% Diquat

Figure 2.2 DYNAMIC SURFACE TENSION MEASUREMENTS
0.5% Diquat solutions with NEWKALGEN




concentrations are expressed as percent weight/volume

Figure 2.3


DYNAMIC SURFACE TENSION MEASUREMENTS
0.5% Diquat solns. with DICLOFOP-METHYL
Surface Tension (mN/m)
75
70
65
60
55
50
45
40
35
30
0
5
10
15
20
25
30
35
162
70
39
23
14.5
9.5
T (ms)
Frequency (Hz)
0.01% Diclof +0.5% Diquat
0.032% Diclo +0.5% Diquat
0.1% Diclofo +0.5% Diquat
0.5% Diquat
concentrations are expressed as percent weight/volume

# 6. Experiment 3

# 6 EXPERIMENT 3 The Influence of Ethofumesate on the Wettability of Poppy and Weed Leaves.

## 6.1 Introduction

It has been established from the previous experiments that diclofop-methyl has certain properties of a surfactant (ie it can reduce the interfacial and surface tension of a liquid), and a significant difference exists between the wettability of poppy plants and weed species. However, no consideration has yet been given to the role of the first herbicide treatment (asulam/ethofumesate) on the selectivity achieved with the second treatment (diquat/diclofop-methyl). The ability of ethofumesate to inhibit the deposition of epicuticular waxes has been demonstrated by a number of workers (Leavitt *et al.*, 1978; Duncan *et al.*, 1981 and Duncan *et al.*, 1982), and has been discussed at length in the literature review. As the contact angles examined in Experiment 1 represent a surface phenomenon, and ethofumesate possibly alters this surface, these angles are re-examined after subjecting the plants to ethofumesate.

The effect of leaf age in influencing spray retention is also examined. For as reported in the literature review, leaves can become progressively easier or harder to wet with age depending on the rate of epicuticular wax deposition and leaf expansion.

Therefore, through the use of contact angle measurements, it is the aim of this experiment to investigate the effect of ethofumesate on epicuticular wax formation on both young and old leaves.

## 6.2 Materials and Methods

Only those plant species which were classified as 'hard' to wet in Experiment 1 (ie poppy, field poppy, fumitory and fat hen) were used in this investigation. The growing conditions for these plants was the same as for those plants examined in the first experiment. Five weeks from the date of sowing the plants were sprayed with water (control), asulam, ethofumesate and asulam/ethofumesate. No surfactant was used with these sprays. By spraying the plants with the conventional herbicide treatment and the individual components of this treatment, it was possible to establish which herbicide is influencing contact angles.

Contact angles were measured using the same procedure as outlined in Experiment 1. It was not the aim of this experiment to examine the effects of different surfactants or surfactant concentrations on contact angles, and as such measurements were only made with diquat (0.5%) and diquat (0.5%) + diclofop-methyl (1.0% v/v). This explains why the contact angles that appear in this experiment are higher than those that were presented in Experiment 1.

It should be noted that in this experiment the term, 'old' leaf refers to any fully expanded leaf at the time of spraying, while a 'young' leaf represents any developing leaf present at the time of spraying.

## 6.3 Results and Discussion

The effect of ethofumesate and asulam on contact angle measurements can be seen in Tables 6.1 to 6.4. All results and statistical analysis are included in Appendix 3.

It can be seen that there was a pronounced difference between contact angles recorded on poppy and weed leaves for the diquat compared with the diquat + diclofop-methyl treatments. This was apparent for both old and young leaves. In previous work, it was established that diclofop-methyl can act as a surfactant. This experiment confirms the surfactant properties of diclofop-methyl.

In examining the effects of asulam and ethofumesate, on poppy (Table 6.1), there was no significant difference between these herbicide treatments and the control on young leaves. This applied to measurements made with both diquat and diquat + diclofop-methyl. On older leaves it can be seen that asulam does not differ significantly from the control. Yet, both the ethofumesate and ethofumesate/asulam treatments significantly reduced contact angles when measured with both diquat or diquat + diclofop-methyl. Ethofumesate has only been reported to inhibit epicuticular wax synthesis during leaf expansion (Leavitt *et al.*, 1978; Duncan *et al.*, 1981 and Duncan *et* *al.*, 1982). From the results of this experiment, it is proposed that epicuticular wax synthesis is inhibited only on the expanded leaves of poppy plants, and/or these epicuticular waxes are more easily cast off, by ethofumesate treatment only once the leaf is fully expanded. At this stage this is only an assumption, however, one can rightfully contend that an increase in herbicide retention, and hence damage, will occur if diquat sprays follow ethofumesate applications when there is high proportion of old to young leaves.

**Table 6.1** Response of poppy to ethofumesate/asulam. (units: degrees)

| | Young | | Old | |
|---|---|---|---|---|
| | diquat | diq/d.-methyl | diquat | diq/d.-methyl |
| Control | 135 | 61 | 136 | 64 |
| Asulam | 134 | 56 | 127 | 59 |
| Ethofumesate | 131 | 61 | 117 | 55 |
| Ethofumesate/Asulam | 131 | 60 | 111 | 42 |

**L.S.D = 8.14 (t=0.05)**

The response of field poppy to the treatments is presented in Table 6.2. This plant responded similarly to poppy, in that there was no significant effect of ethofumesate on young leaves. On older leaves, however, there was a significant effect of ethofumesate/asulam only when diquat + diclofop-methyl was applied. As with poppy, it is proposed that epicuticular wax synthesis is inhibited only on expanded leaves, and/or these epicuticular waxes are more easily cast off, by ethofumesate treatment only once the leaf is fully expanded.

**Table 6.2** Response of field poppy to ethofumesate/asulam. (units: degrees)

| | Young | | Old | |
|---|---|---|---|---|
| | diquat | diq/d.-methyl | diquat | diq/d.-methyl |
| Control | 128 | 61 | 132 | 55 |
| Asulam | 127 | 61 | 128 | 58 |
| Ethofumesate | 125 | 62 | 127 | 54 |
| Ethofumesate/Asulam | 125 | 67 | 127 | 44 |

**L.S.D = 8.14 (t=0.05)**

As seen in Tables 6.3 and 6.4 fumitory and fat hen responded in the same manner to the ethofumesate/asulam treatments. However, unlike poppy and field poppy there was no significant effect of ethofumesate treatments on old leaves of these plants. This observation applied to measurements made with both diquat and diquat + diclofop-methyl.

**Table 6.3** Response of fumitory to ethofumesate/asulam. (units: degrees)

| | Young | | Old | |
|---|---|---|---|---|
| | diquat | diq/d.-methyl | diquat | diq/d.-methyl |
| Control | 129 | 62 | 120 | 53 |
| Asulam | 130 | 60 | 126 | 61 |
| Ethofumesate | 107 | 37 | 127 | 60 |
| Ethofumesate/Asulam | 100 | 40 | 126 | 61 |

**L.S.D = 8.14 (t=0.05)**

**Table 6.4** Response of fat hen to ethofumesate/asulam. (units: degrees)

| | Young | | Old | |
|---|---|---|---|---|
| | diquat | diq/d.-methyl | diquat | diq/d.-methyl |
| Control | 129 | 54 | 132 | 54 |
| Asulam | 130 | 55 | 131 | 56 |
| Ethofumesate | 111 | 40 | 132 | 59 |
| Ethofumesate/Asulam | 104 | 40 | 132 | 60 |

**L.S.D = 8.14 (t=0.05)**

From the response of young leaves of fumitory and fat hen plants, it is apparent that there was a significant reduction in contact angle on the ethofumesate treated plants. Yet, there was no effect of asulam on either plant. From this it is possible to conclude that ethofumesate alters the surface characteristics of the young leaves of fumitory and fat hen plants. Thus, after application of ethofumesate the developing leaves of these 'hard' to wet weeds will become easier to wet with diquat solutions.

Statistical analysis in Appendix 3 reveals that a significant interaction exists between leaf surface age and the ethofumesate/asulam treatment ($p = 0.0001$). As discussed in the literature review, ethofumesate has been found to inhibit the deposition of alkane ($C_{29}$) and sec-ketone ($C_{29}$) components of surface waxes of developing leaves (Leavitt *et al.*, 1978 and Duncan *et al.*, 1982). These reports have only focussed on cabbage and sugar beet. It is inevitable that as a wider range of species are studied the potential of ethofumesate to inhibit, or even increase, the deposition of individual wax components will be realised. Primary alcohols have not been isolated from the surface waxes of cabbage and sugar beet (Holloway, 1969), however, they have previously been found to be inhibited after herbicide treatment of pea plants (Still *et al.*, 1970). Duncan *et al.*, (1981) found that ethofumesate is rapidly absorbed and extensively accumulated in untreated plant components of fat hen plants when at the seedling (two leaf) stage. Therefore, after accumulation, the deposition of the primary alcohol, and possibly the aldehyde wax fractions of fat hen, identified by Taylor *et al.*, (1981), may be inhibited in young leaves upon treatment with ethofumesate. The wax components of fumitory leaves could not be identified from the literature, however, from the results of this experiment it is conceivable that the epicuticular wax fraction of the leaves of this plant are also inhibited by ethofumesate treatment.

Therefore, it is proposed that ethofumesate, by disrupting the surface characteristics of the young leaves of fumitory and fat hen, effectively widens the margin of herbicide selectivity achieved with the current herbicide program.

The results have also indicated that the old leaves of poppy, and field poppy, are effected by ethofumesate. It is possible, that as with poppy, the epicuticular wax fraction of field poppy contains a large proportion of secondary alcohols, identified by Holloway *et al.*, (1976). It is hypothesised, that unlike other plant species epicuticular wax deposition continues on poppy and field poppy leaves up to, and after leaf expansion. However, this deposition is inhibited by ethofumesate treatment only *after* the leaf is fully expanded. A possible reason for this is that ethofumesate may be selectively translocated from young to developed leaves for degradation after foliar uptake. This is feasible after considering that Duncan *et al.*, (1981) found species tolerant to ethofumesate, were capable of inactivating ethofumesate to organic-soluble metabolites after acropetal translocation. Although photosynthesis and dark respiration were initially inhibited in tolerant species, after inactivation these plants recovered rapidly.

As will be illustrated in the field trial results, poppies are desiccated only slightly by the second spray, and unlike the weed species are able to recover. The desiccation may be a response to the initial damage incurred from the first spray application, but as the poppies are able to quickly regain photosynthetic and respiratory activities after the ethofumesate treatment, they are not as sensitive to future applications of diquat/diclofop-methyl compared with fat hen and fumitory.

# 7. Experiment 4

# 7 EXPERIMENT 4 Examination of Leaf Epicuticular Waxes.

## 7.1 Introduction

Part A of this experiment reports on the effect of ethofumesate on the morphology of young and old leaf surfaces and discusses some possible implications in regards to future herbicide applications. In Part B, a chemical analysis of the wax of the species investigated in Part A, has been undertaken to determine if the chemical composition of leaf waxes can be related to the mechanism of ethofumesate action.

## Part A Wax Morphology

### 7.1.a Materials and Methods

The nature of the adaxial leaf surface of selected weed and poppy species was observed by scanning electron microscopy (SEM). The seedlings were subjected to a combined application of ethofumesate and asulam at rates of 1 l/ha and 5 l/ha respectively. After six days from the date of spraying the first fully expanded leaf and a young developing leaf were examined (nb the stages described apply to the time of spraying). The control consisted of two components; firstly the examination of expanded and developing leaves from those plants treated with deionised water, and secondly the inspection of these leaves after washing in chloroform for 10 to 15 seconds to remove any wax (see Plate 9.0).

Rectangular leaf pieces (4x8 mm) were cut from the leaf at one side of the mid-vein near the leaf centre. The leaf pieces were mounted, abaxial surface down, onto SEM brass stubs using double sided adhesive tape. Specimens were then sputter-coated with 20-25 nm of gold (ultra pure) in an argon atmosphere using a Balzers Union sputter device. Coated specimens were then examined with a Phillips 505 scanning electron microscope operated at an accelerating voltage of 20 kV in high resolution mode. Photomicrographs were taken on Ilford FP4 Plus 125 (120) film using a scan duration of 1000 lines/frame, a line time of 32 msec and a spot size of 50 nm.

The entire process took less than 15 minutes from the time the leaf pieces were first cut.

### 7.2.a Results and Discussion

Scanning electron micrographs of the leaf surfaces are shown in Plates 5.0 to 8.0. These micrographs are selections, based on clarity, taken from two replicates. In general the wax morphology differed widely between species. The main features of the different leaf surfaces can be summarised:

Plate 5.0) Poppy: Wax morphology is comprised of a random arrangement of small aggregates on both young and old leaves. Similar results have been reported for poppy by Baker and Parsons, (1971). There appears to be no effect of leaf age on the surface morphology. Likewise, there was no visible effect of the ethofumesate treatment on old or young leaves.

Plate 6.0) Field Poppy: As with poppy, the wax deposits on the adaxial leaf surface of field poppy consist of a random arrangement of granular aggregates. There was no discernible change in these wax structures after application of ethofumesate. On inspection of young leaves, it appears that the control leaf is comprised of a composite arrangement of filaments and granular aggregates. It is difficult to establish whether this is an effect of age, or a result of beam damage during the magnification process. According to Baker and Holloway, (1971) under high magnification (ie > x 5,000) waxy leaf surfaces may blister or melt, therefore, disrupting the leaf surface.

Plate 7.0) Fumitory: On the adaxial surface of fumitory leaves, the wax crystals form long round rodlets that progress outwards from the leaf surface. The rodlets, which appear to be tubular and solid, are of fairly uniform length and there is little evidence of branching. Each rodlet appears to be exuded at the leaf surface from a common base. On comparing the control leaves it is apparent that there was no effect of leaf age on these wax crystals. However, there was a marked inhibition in epicuticular wax synthesis and/or extrusion on those leaves of young plants treated with ethofumesate.

Plate 8.0) Fat Hen: In contrast to the previously examined plants, the adaxial leaf surface of fat hen was covered by small semi-circular wax platelets, which primarily project vertically from the cuticle surface. In comparing the control leaves, it appears that these wax structures have not yet fully developed on the immature leaves. There was no visible reduction in the quantity of surface wax on either young or old leaves after treatment with ethofumesate. However, on close examination of the old leaf surface of plants treated with ethofumesate, the edges of the wax platelets have a perforated appearance. Once again, it is difficult to predict if this observation was simply the result of damage during the magnification process, or in fact a real treatment effect. However, as the entire leaf surface is still covered by the wax platelets, a change in leaf wettability would not be expected.

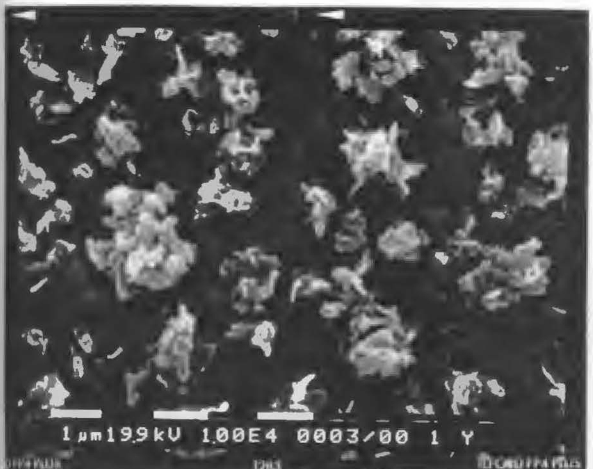
Plate 9.0) Control: A control leaf surface from each plant species was washed in chloroform to remove any wax. The leaf surface presented here, a fumitory leaf, was typical of all the control leaves; ie a smooth, featureless cuticle.

From these results it appears that epicuticular synthesis and/or deposition is only inhibited on the young developing leaves of fumitory. This supports the findings made in the previous experiment that there was a significant reduction in the contact angle, made by diquat solutions, on those young leaves of fumitory plants treated with ethofumesate. In regards to the fat hen and field poppy, although there were changes in the fine structure of wax deposits, there was no visible change in leaf surface coverage. According to Holloway (1969), Baker and Bukovac (1971) and Stevens and Baker (1987) provided the entire leaf surface is covered with wax deposits, the amount of wax present is unimportant when considering leaf wettability. Therefore, as with poppy, it is not possible to correlate those changes in contact angles determined in the previous experiment with epicuticular wax deposition on these plants.

**Plate 5.0**

**Poppy**

Control | Ethofumesate/Asulam





Adaxial young leaf

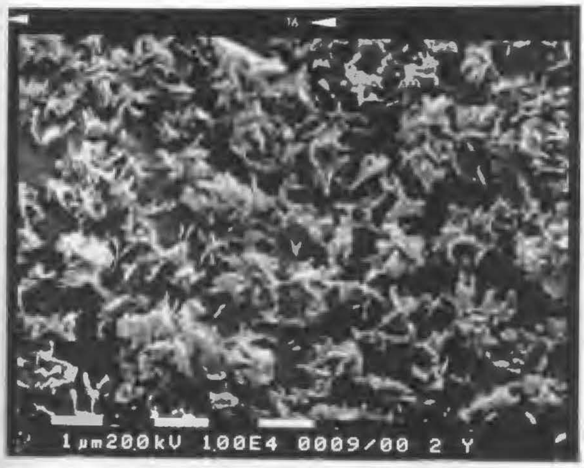


Adaxial old leaf

**Plate 6.0**

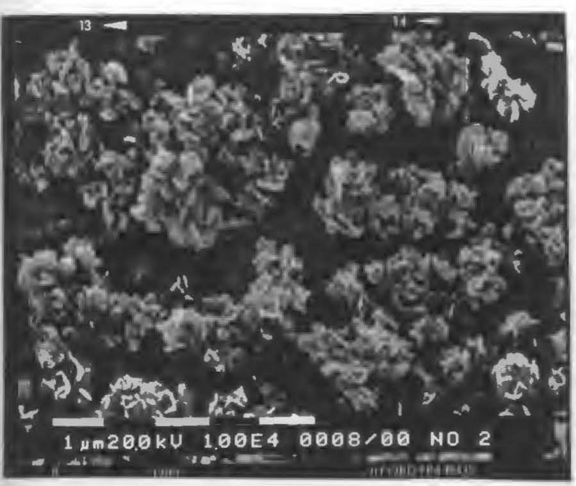
**Field Poppy**

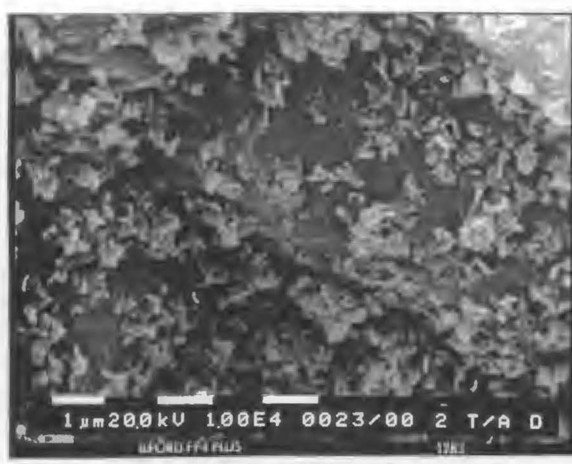
<u>Control</u> | <u>Ethofumesate/Asulam</u>





Adaxial young leaf




Adaxial old leaf

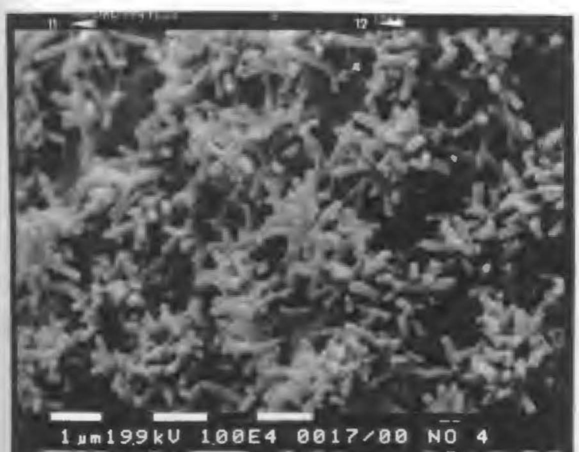
## Plate 7.0

## Fumitory

Control | Ethofumesate/Asulam



Adaxial young leaf



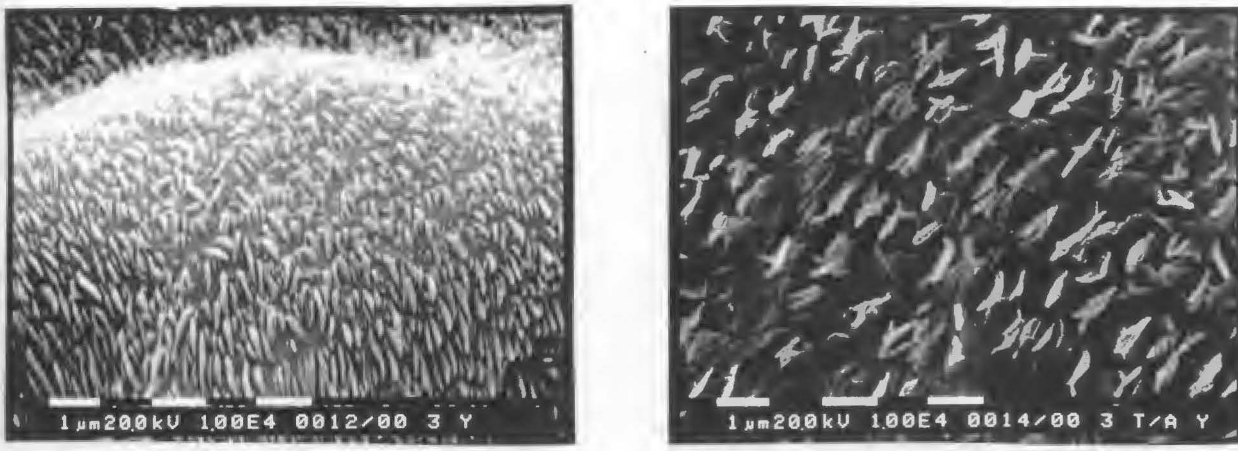


Adaxial old leaf

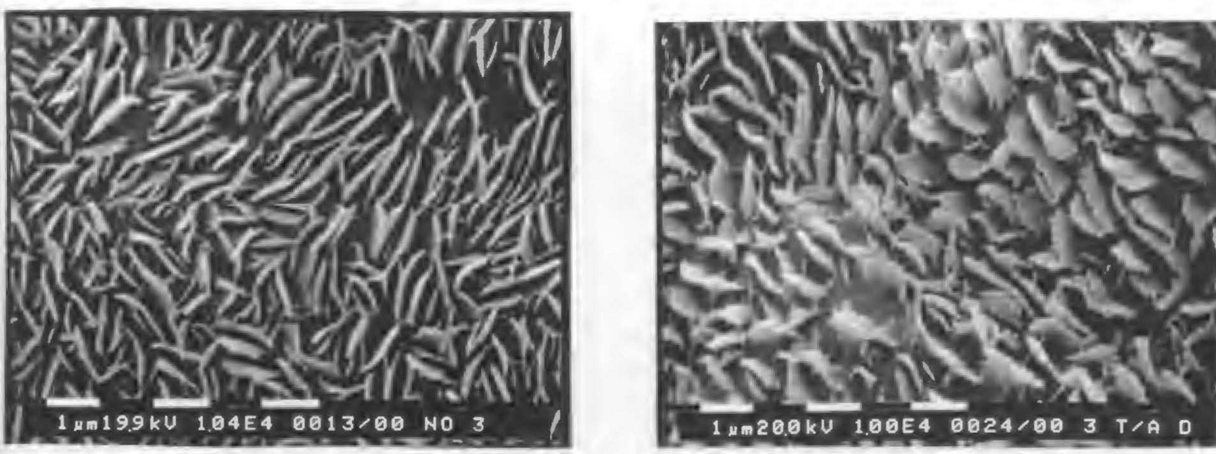
## Plate 8.0

## Fat Hen

Control | Ethofumesate/Asulam



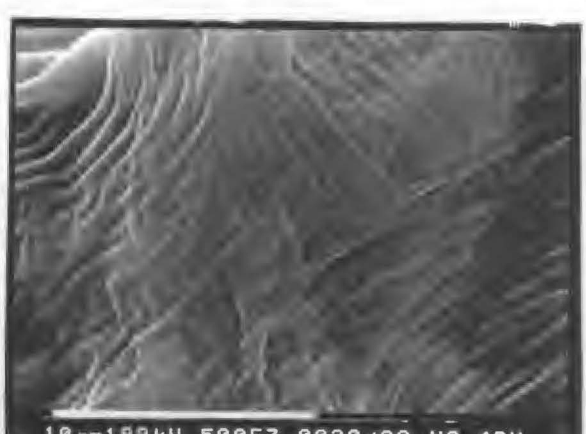

Adaxial young leaf




Adaxial old leaf

## Plate 9.0

## Control




Adaxial surface of fumitory leaf treated with chloroform.

## Part B Wax Chemistry

### 7.1.b Materials and Methods

In addition to removing the epicuticular waxes from those plants exposed to the combined application of ethofumesate and asulam, epicuticular waxes were also removed from plants which were subjected to separate treatments of ethofumesate and asulam at rates of 1 l/ha and 5 l/ha respectively. In each treatment, fresh leaf material was used. Preparation of samples involved carefully removing fully expanded or young developing leaves and measuring individual leaf areas using a planimeter. The leaves were then allowed to wilt slightly to ensure stomatal enclosure. Each leaf was then immersed in approximately 20 ml of redistilled chloroform in individually labelled vials. After immersion, the leaf was gently agitated, using a vortex mixer for 30 to 60 seconds.

Initially attempts were made to remove the epicuticular waxes of untreated poppy leaves by washing the leaves in hexane. However, on analysis it was revealed that this solvent did not completely remove waxes from the surface of poppy leaves. On reviewing the literature on techniques used to remove epicuticular waxes Michaich (1989) found that although hexane has been used in the past to isolate epicuticular waxes with some success, chloroform is the most commonly used solvent for this purpose. This is due to its ability to remove all components of the epicuticular wax, therefore forming the only valid sample of the waxes present. This finding was supported by SEM analysis of chloroform treated leaves (see Plate 9.0).

The chloroform-wax solution was evaporated under a forced air stream, and the wax extract stored as a dried sample until analysis. Wax removal, evaporation and storage took place at room temperature. Prior to GC-MS analysis the wax was redissolved in approximately 1 ml of redistilled chloroform. Analysis of the crude extract (as a chloroform solution) and identification of components were performed by GC-MS on a 25m x 0·3mm x 0·17μm film thickness Hewlett Packard (HP) column using a HP-5890

GC with open slit interface at 300°C. Scanning was performed from mass 500 to 40 at 1·4 scan/second. The oven temperature was programmed from 30°C to 240°C at 30°C/minute, 240°C to 300°C at 10°C/minute and held at 300°C for 8 minutes. Helium was used as the carrier gas.

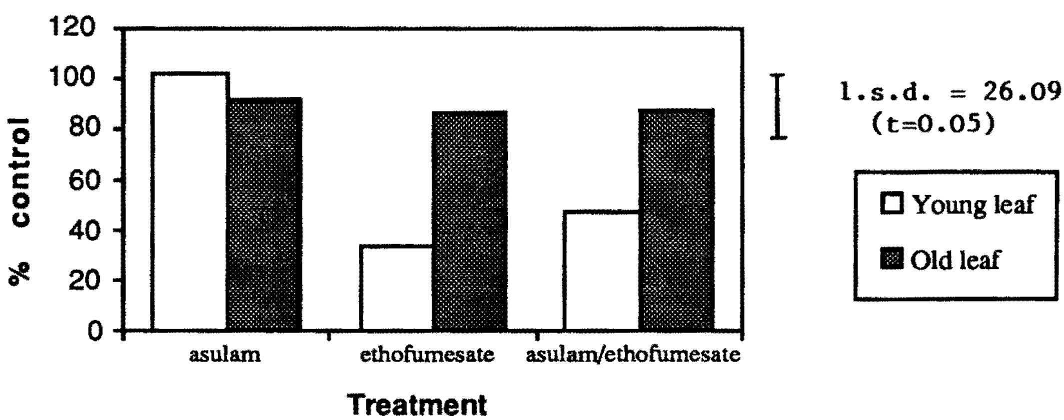
Quantitative measurements of components were determined by GC-MS using automatic sample injection on an identical capillary column and a HP-5970 mass selective detector linked to a HP integration system, and are based upon integrated peak areas using octadecane as an internal standard.

The data is expressed in a wax units/$cm^2$ leaf area basis, and expressed as a percentage of control. Initially, only a single determination was made for each treatment, however, seven days after the first analysis (ie 12 days post spraying) two further replicates were completed. In each replicate, three leaves were harvested and combined. Changes in wax composition are assessed by comparison of area of the peaks of interest, relative to the internal standard.

### 7.2.b Results and Discussion

Foliar applied ethofumesate treatments significantly decreased the amount of epicuticular wax on the surface of young fumitory leaves (Figure 7.1). There was no significant difference observed by the herbicide treatments on old leaves.

**Figure 7.1**: Influence of herbicide treatment on epicuticular wax deposition on fumitory.



The above data supports the findings made in the previous experiments, that ethofumesate alters the surface characteristics of young leaves. Epicuticular waxes are deposited only during leaf expansion, (Kolattukudy, 1980), therefore, once developed, these leaves will have a reduced coverage of surface wax compared with untreated leaves. This, combined with the findings made by Duncan *et al.*, (1982) that ethofumesate decreases metabolism and inhibits photosynthesis in susceptible species, can account for why fumitory, which was classed as 'hard' to wet after the first experiment, is selectively controlled by diquat + diclofop-methyl following asulam/ethofumesate treatment.

The major components of the fumitory leaf wax were determined by GC-MS and there was no observable differences between young and old leaves. The major components (Figure 7.2) were identified as a C-26 primary alcohol, a C-28 primary alcohol and a C-30 aldehyde. As illustrated in Figure 7.3, after ethofumesate treatment of young leaves, there was a decrease in both the C-26 and C-28 primary alcohol wax components, relative to the internal standard, while the C-30 aldehyde was absent. Therefore, ethofumesate appears to be an inhibitor of C-26 and C-28 primary alcohols and C-30 aldehydes.

Although not as pronounced as in fumitory, fat hen responded similarly to ethofumesate treatments. As seen in Figure 7.4, there was a significant reduction in the amount of epicuticular wax on the surface of young leaves treated with ethofumesate, however, there were no significant difference observed on old leaves.

Figure 7.2 Fumitory : wax chemistry of young leaves.

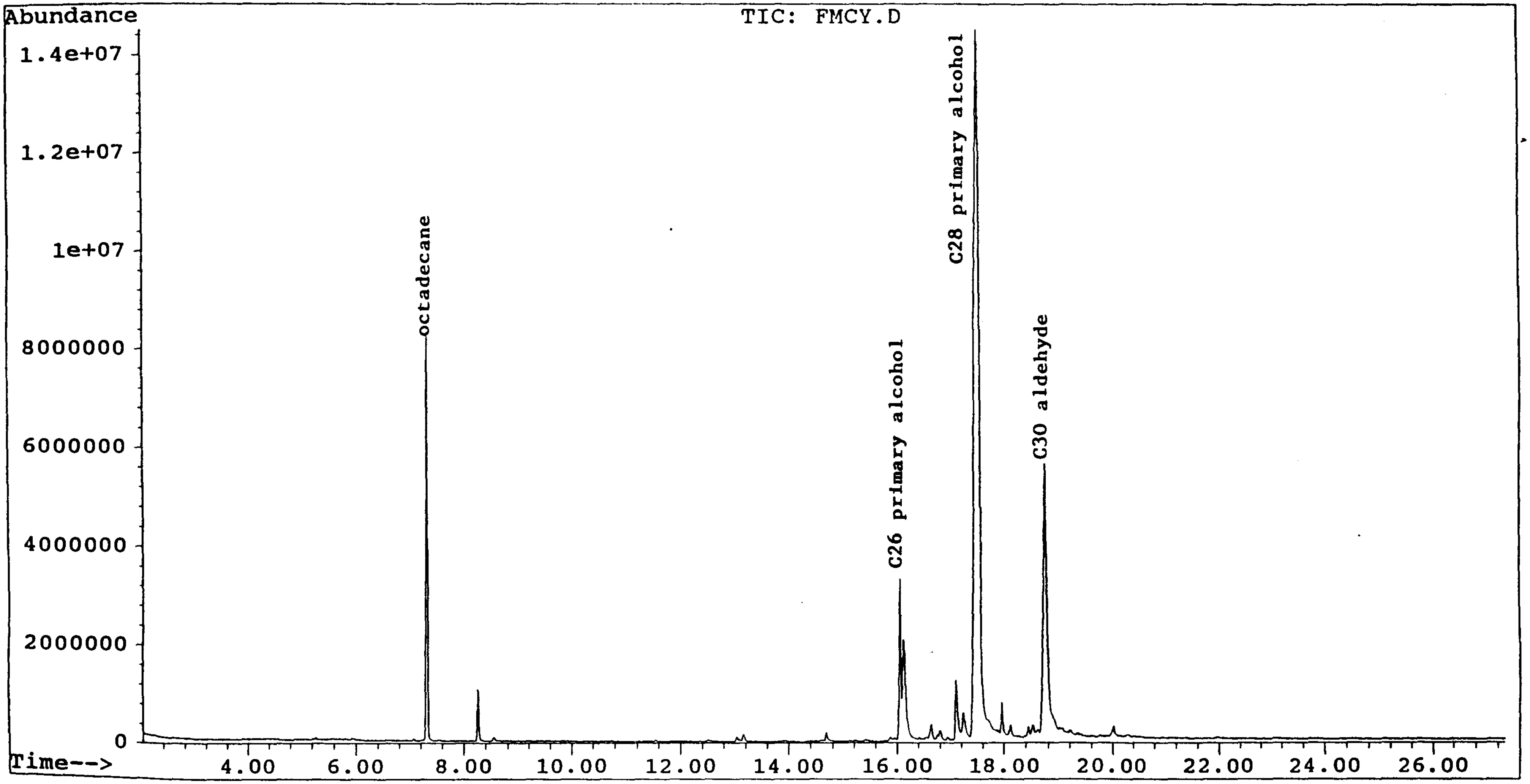


Figure 7.3 Fumitory : wax chemistry of young leaves after ethofumesate treatment.

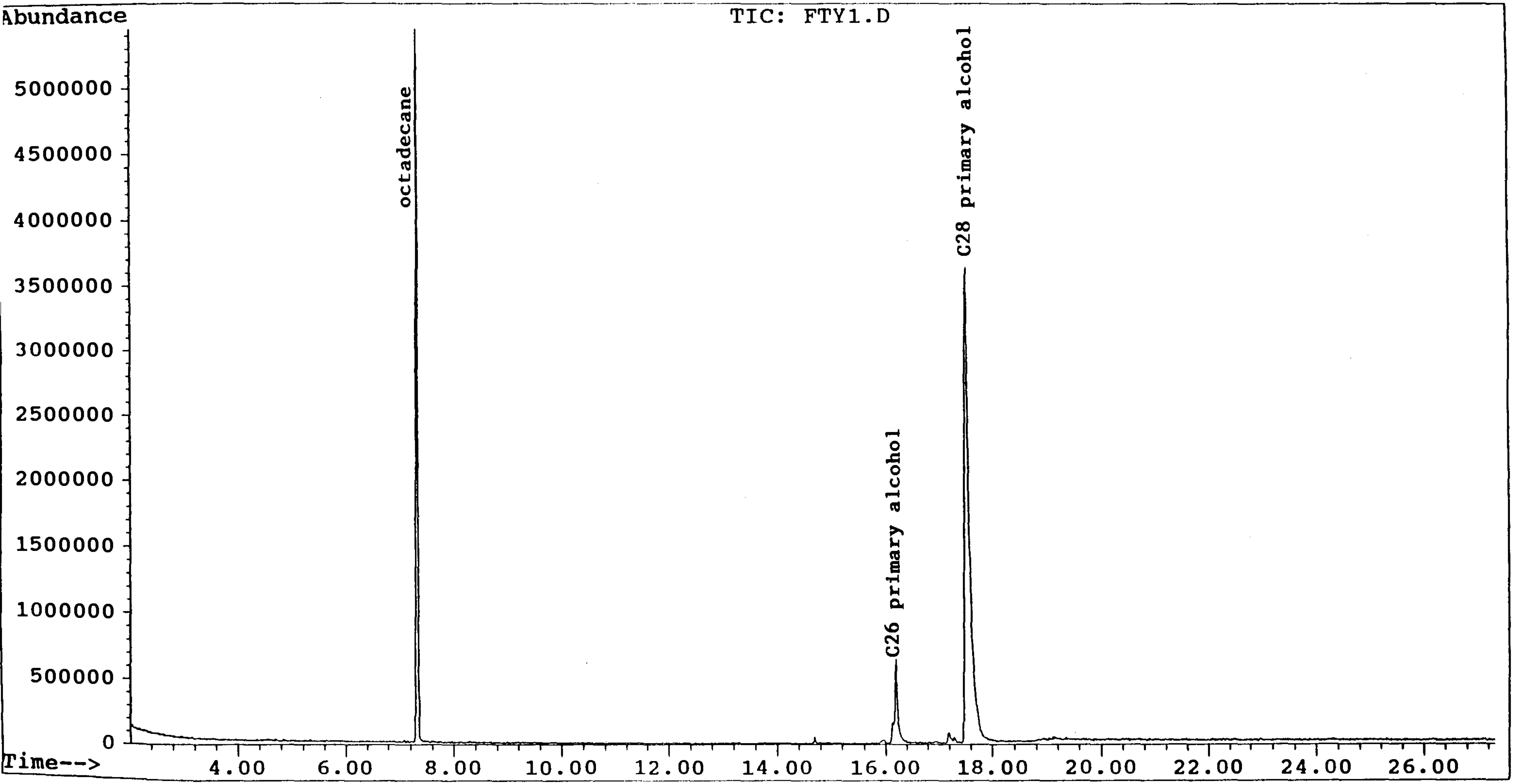
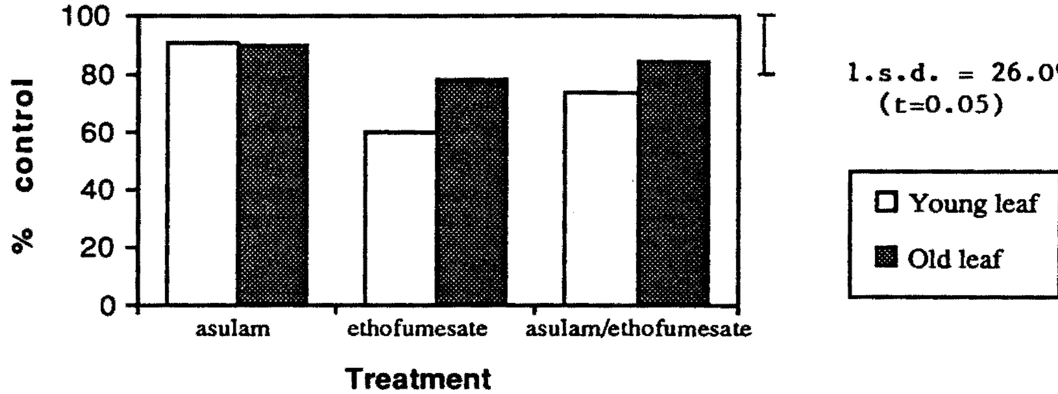


**Figure 7.4:** Influence of herbicide treatment on epicuticular wax deposition on fat hen.




Although these findings are not in agreement with the observations made in Part a of this experiment, they do support the hypothesis put forward in the previous experiment, that ethofumesate treatment increases spray retention on young leaves due to decreased epicuticular wax formation.

The major components (Figure 7.5) of fat hen waxes were identified as a C-27 alkane, a C-26 aldehyde, a C-26 primary alcohol, a C-28 aldehyde, a C-28 primary alcohol, a C-28 primary alcohol acetate, a C-30 aldehyde and long chain esters. These components were detected on young and old leaves. These components have also been identified by Taylor *et al.*, (1981), who reported that the surface wax of fat hen consisted primarily of aldehydes, primary alcohols and esters. As illustrated in Figure 7.6, ethofumesate treatment of young leaves inhibited, relative to the internal standard, the synthesis of the C-27 alkane, C-26 primary alcohol, C-28 primary alcohol, C-28 primary alcohol acetate and C-30 aldehyde wax components.

Figure 7.5 Fat Hen : wax chemistry of young leaves.

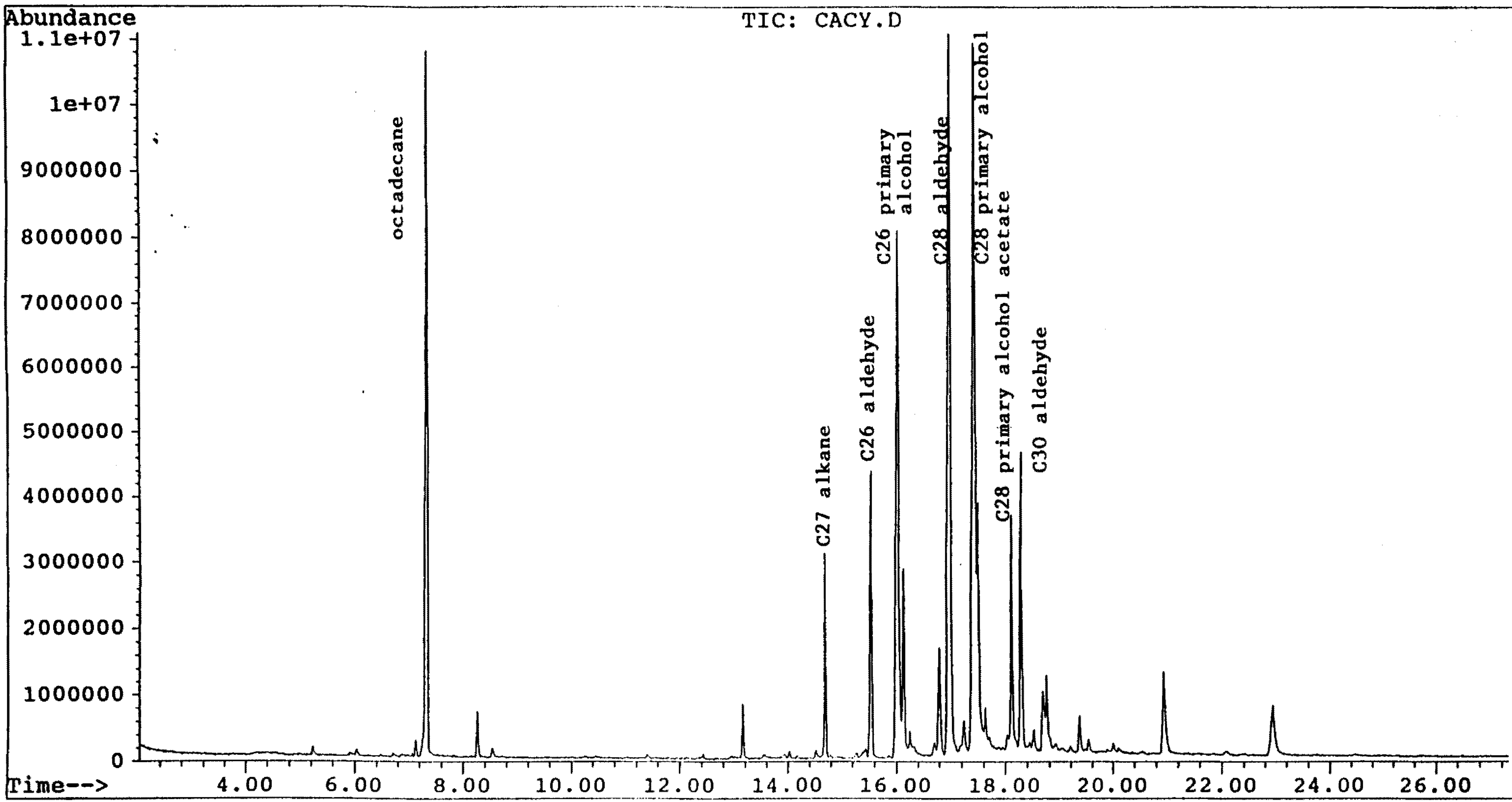
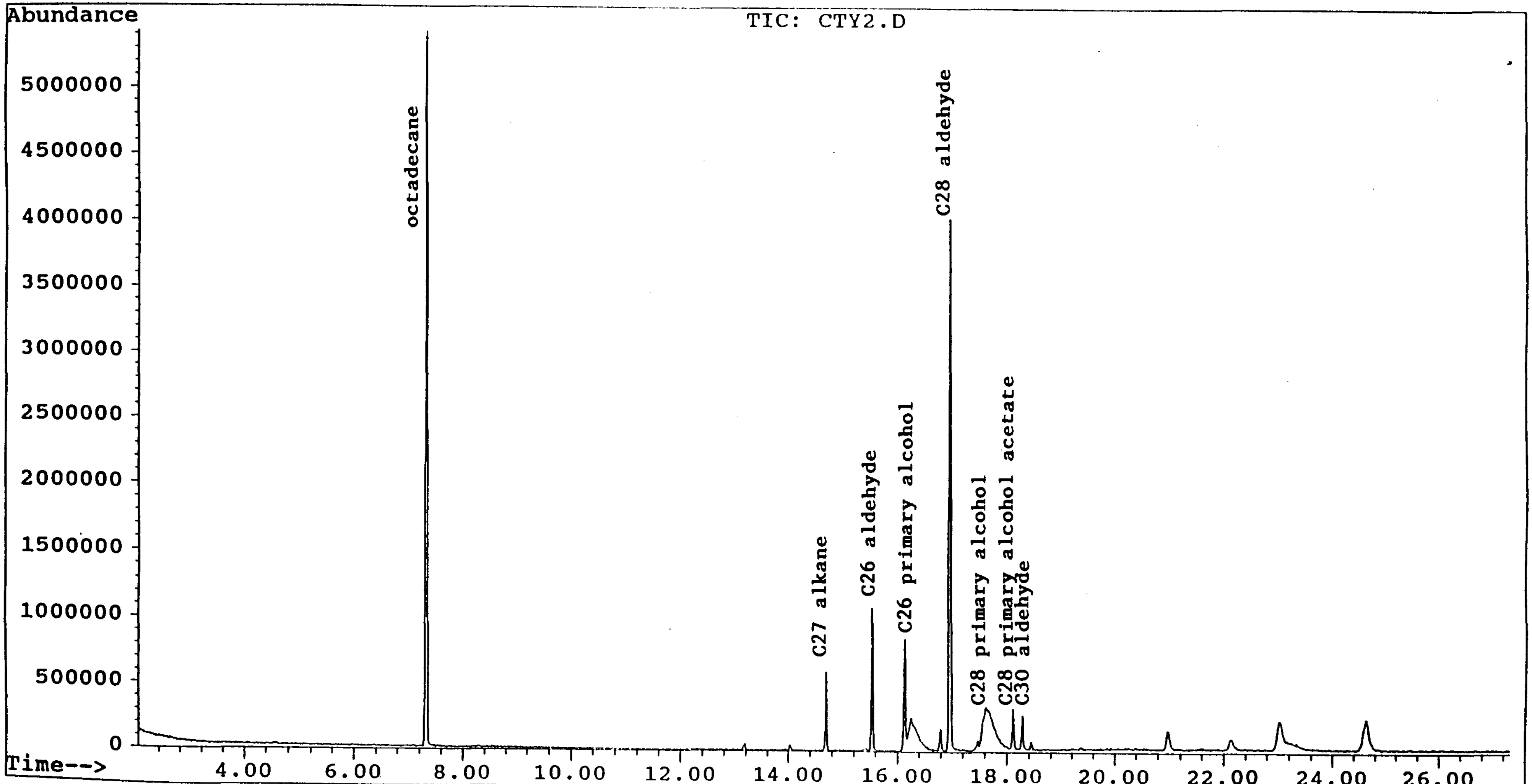


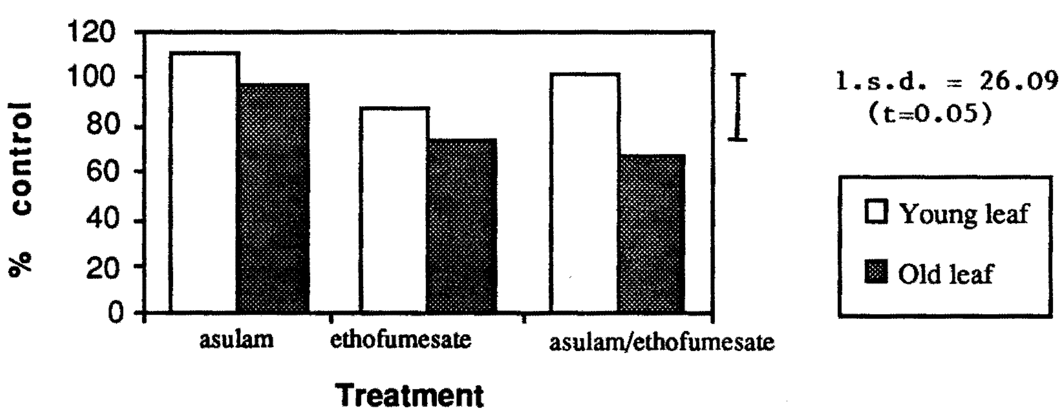
Figure 7.6 Fat Hen : wax chemistry of young leaves after ethofumesate treatment.



This is in accordance with the findings made on examination of fumitory leaves, that primary alcohols and long chain aldehydes are inhibited by ethofumesate. Primary alcohols have previously been reported to be inhibited only on pea leaves after diallate treatment (Still *et al.*, 1970), and no literature exists to explain the inhibition of aldehydes in response to herbicide treatment. However, Leavitt *et al.*, (1978) and Duncan *et al.*, (1982) have reported decreased alkane deposition on cabbage and sugar beet after ethofumesate treatment.

As demonstrated in Figure 7.7, there was no significant effect of herbicide treatments on the young leaves of field poppy. However, wax deposition on the old leaves of those plants subjected to the asulam/ethofumesate was significantly reduced.

**Figure 7.7:** Influence of herbicide treatment on epicuticular wax deposition on field poppy.




It should be noted that a similar result was observed in determining contact angles of asulam/ethofumesate treated plants. There it was proposed that wax deposition was only inhibited once the leaves were fully expanded. The major wax components (Figure 7.8) of young and old field poppy leaves are identified as a C-26 primary alcohol, a C-28 aldehyde a C-29 -10-ol (a secondary alcohol), and according to Davies (1993, pers. comm.) an 'unidentifiable' component. As illustrated in Figure 7.9, asulam/ethofumesate treatment of old leaves inhibited, relative to the internal standard, the deposition of the C-26 primary alcohol and the 'unknown' wax components.

**Figure 7.8 Field Poppy : wax chemistry of old leaves.**

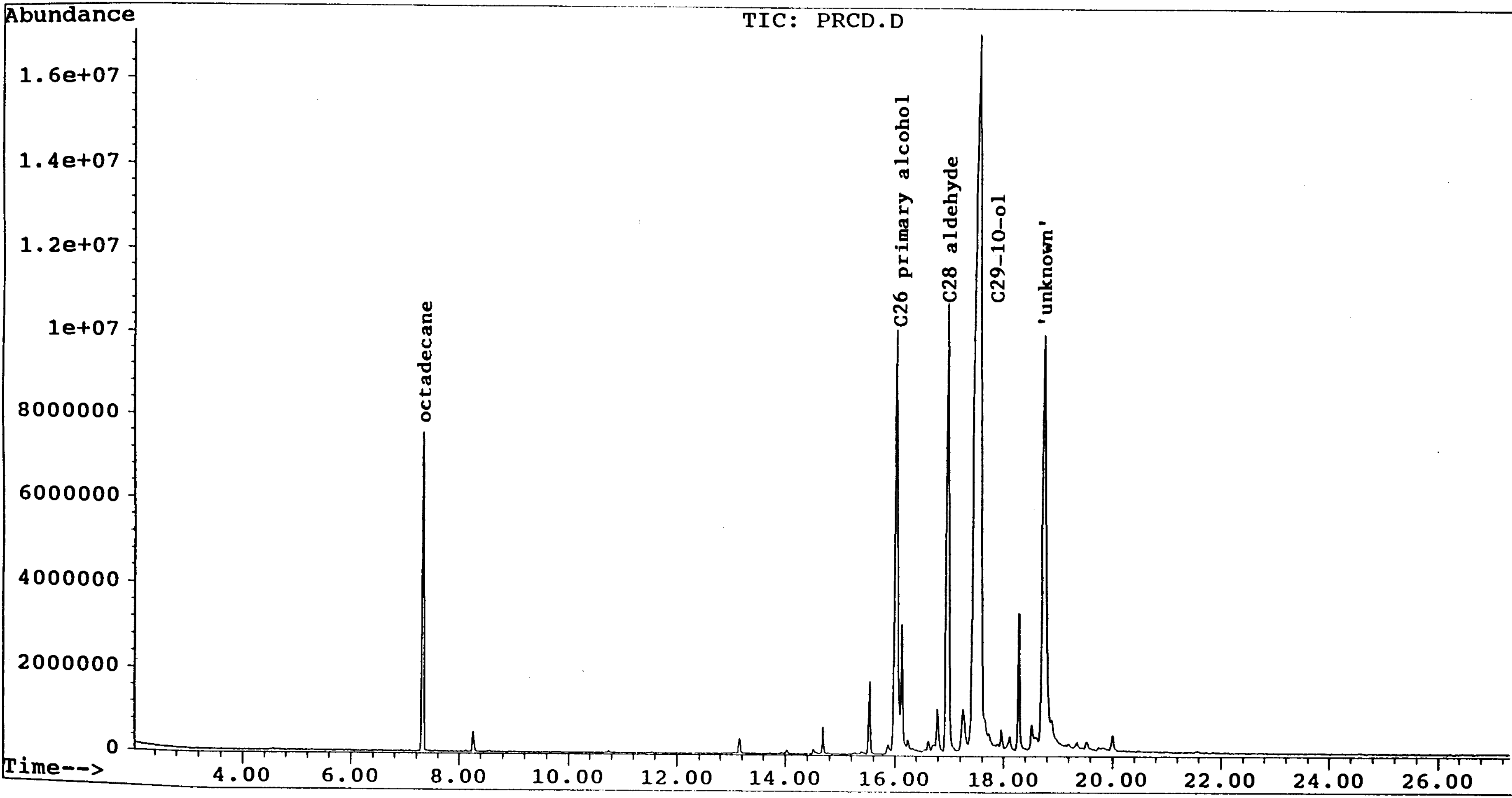
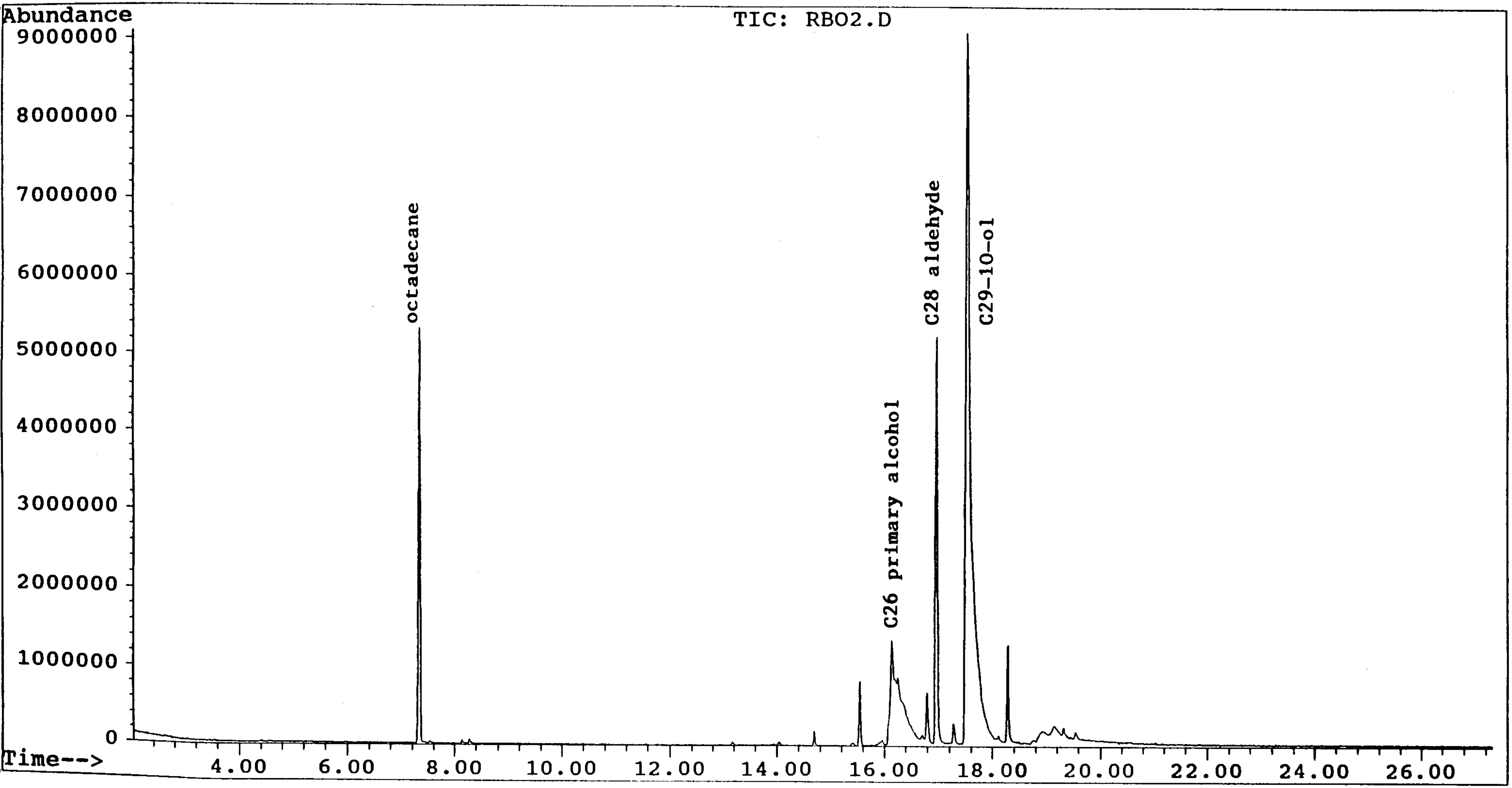


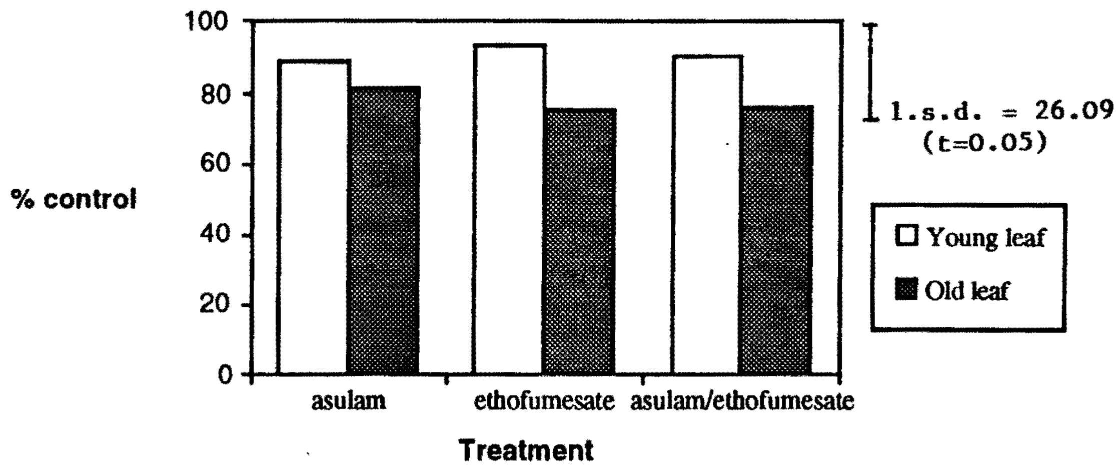
Figure 7.9 Field Poppy : wax chemistry of old leaves after ethofumesate treatment.



This supports the concept that wax deposition is being inhibited on old leaves, a phenomenon yet to be reported in respect to the effects of ethofumesate. However, it was also observed that these wax components were inhibited on young leaves of ethofumesate treatments. Therefore, the inhibition of these waxes can not be solely responsible for the reported significant effect in Figure 7.7, or the difference between young and old leaves suggested by contact angle measurements.

Foliar applied ethofumesate treatments had no significant effect on the amount of epicuticular wax on the surface of young or old poppy leaves (Figure 7.10).

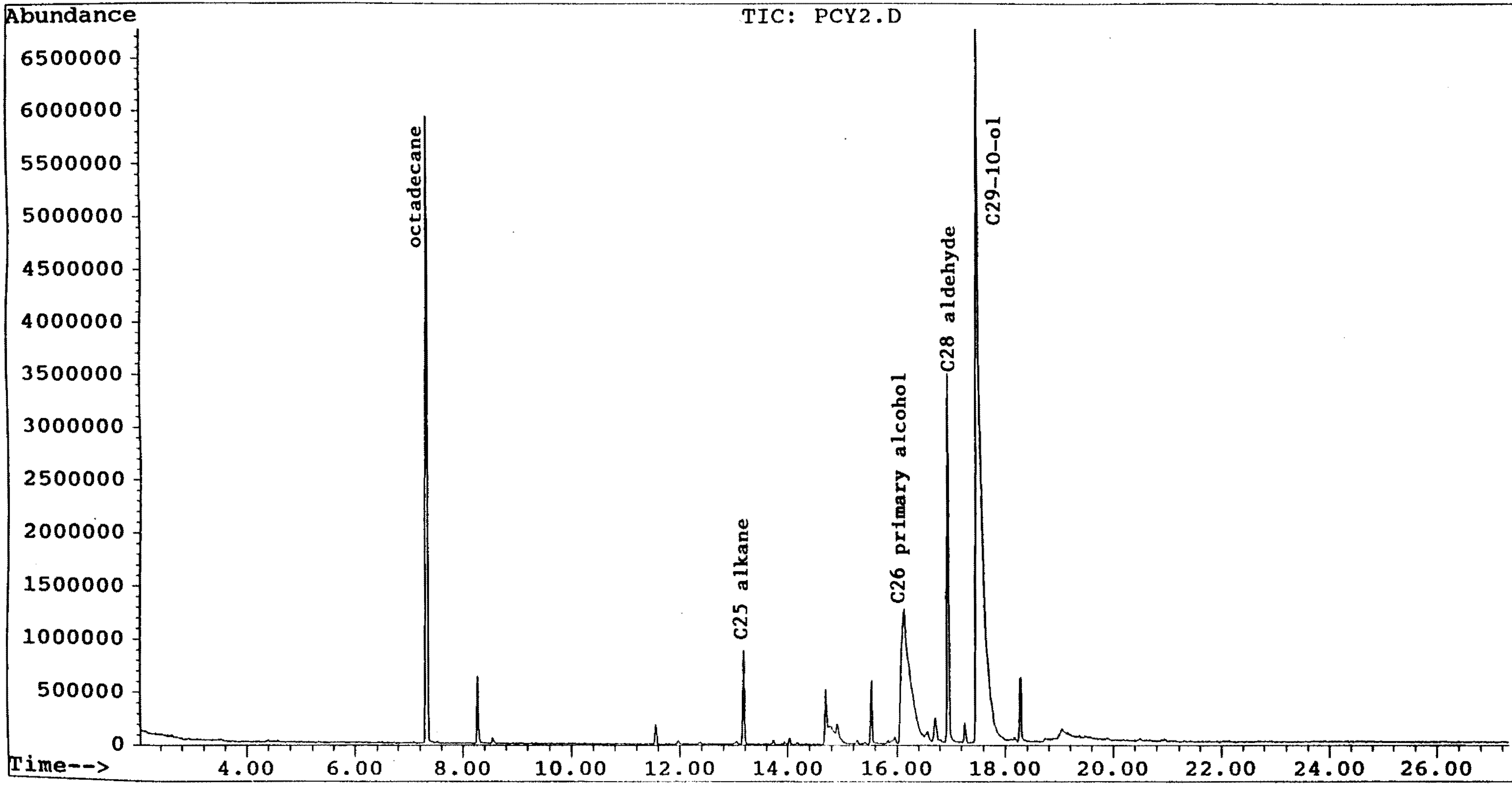
**Figure 7.10**: Influence of herbicide treatment on epicuticular wax deposition on poppy




This supports the observations made in Part a of this experiment, that leaves of poppy plants were not affected by ethofumesate. The major wax components (Figure 7.11) of young and old poppy leaves are identified as a C-25 alkane, a C-26 primary alcohol, a C-28 aldehyde and a C-29-10-ol. As illustrated in Figure 7.11, the C-29-10-ol represents the major wax component, a finding shared by Holloway *et al.*, (1976).

There was no observable change in the wax components of young or old poppy leaves after herbicide treatment. This discounts the hypothesis suggested earlier that epicuticular wax deposition is only inhibited on the old leaves of poppy plants. However, as neither the alkane, aldehyde or primary alcohol wax components were affected by ethofumesate treatment it is reasonable to propose that poppy is tolerant to

Figure 7.11 Poppy : wax chemistry of young leaves.



ethofumesate treatment whilst fumitory, fat hen and to a lesser extent field poppy are susceptible. Inhibition of wax deposition on young leaves, associated with the findings made by Duncan *et al.*, (1982) that ethofumesate decreased metabolism and inhibited photosynthesis in susceptible species, can account for the why fumitory and fat hen, which were classed as 'hard' to wet after the first experiment, are selectively controlled by diquat + diclofop-methyl. This supports the commonly held industry view that the asulam/ethofumesate treatment is a 'softening-up' spray.

# 8. Experiment 5

# 8. EXPERIMENT 5: Field Trial: the measurement of spray retention and efficacy of diquat solutions.

## 8.1 Introduction

The purpose of this trial was to determine if those findings made in the previous experiments, which were conducted in the laboratory using plants grown in a protected glasshouse environment, can be related to field conditions. From the laboratory work it was demonstrated that the nature of the leaf surface can be altered through the use of herbicides, (viz: ethofumesate), however, the nature of the leaf surface can also change in response to environmental factors. For example, Baker (1974) showed that temperature, humidity and light intensity are important in controlling the size, configuration and distribution of epicuticular wax deposits. Plant surfaces can also be subject to weathering which may effect their wettability. Damage may be caused by the rubbing of leaves on the same or neighbouring plants, by rain or by the scarifying effect of wind-borne sand. Thus, environmental factors can also modify the nature of leaf surfaces, and consequently have the potential to change both leaf wettability and herbicide penetration, which in turn will influence a plants response to post emergent herbicides.

From the initial experiments it was clearly established that diclofop-methyl has the ability to act as a surfactant when incorporated with diquat. Diclofop-methyl is formulated as an emulsifiable concentrate, and as speculated in the literature review, it may not be the active constituent that is producing the synergistic interaction with diquat, but rather the organic solvents and adjuvants of the formulated product. It was the objective of this trial to examine this hypothesis.

Next season (1994) a new formulation of diclofop-methyl is to replace the existing formulation (Fist, pers. comm. 1993). It is not the active ingredient, but rather the inert ingredients (eg solvents and adjuvants) which are to be changed. Therefore, if the proposed hypothesis, that the so called inert ingredients are producing the synergistic relationship with diquat, holds true, then it is possible that this altered product may perform differently, compared with the current formulation, when in admixture with diquat. The new formulation may not behave like a surfactant, or on the other hand it may have the ability to act as a strong surfactant, capable of decreasing herbicide selectivity. Clearly these changes have the potential to limit the future use of this herbicide in weed control programs in poppy crops. Therefore, a further aim of this trial is to compare the performance of this new diclofop-methyl formulation, when in association with diquat, to the current diclofop-methyl formulation

## 8.2 Materials and Methods

Two field trials were conducted, the first trial was performed on a commercial crop of poppies on September 27, 1993 at Belmont, Swansea. The second trial was conducted, again on a commercial poppy crop, on October 11, 1993 at Clifton Jersey Stud, Wesley Vale. Each experiment was designed as a randomised complete block with split plots with three replications of twelve treatments; the control (deionised water), diquat, diquat + diclofop-methyl, diquat + the emulsifiable concentrate solution present in the commercial diclofop-methyl formulation (EC; provided by Hoechst Aust. Ltd.), diquat + the new diclofop-methyl formulation (provided by Hoechst Aust. Ltd.) and diquat + Agral®, times two rates of the combined ethofumesate/asulam application (rate 1: plants sprayed with water; rate 2: 1 l/ha ethofumesate and 5 l/ha asulam). Appendix 5 illustrates the experimental design. Both the control and diquat solutions also contained a water soluble, dye rhodamine b (Spray Marker®). The plots measured 10m x 2m. Each crop was at the 6 to 8 true-leaf stage and the weeds mainly at the 4 to 6 true-leaf stage at the time of spraying. The spraying conditions, weed and crop species present at the time of spraying and their densities (plants $m^{-2}$) were as follows:

| | | |
|---|---|---|
| Swansea: | *Papaver somniferum* | 30 |
| | *Fumaria muralis* | 77 |
| | *Chenopodium album* | 8 |
| | *Phalaris aquatica* | 300 |

Other species 60, comprising *Polygonum aviculare, Polygonum convolvulus, Sinapsis arvensis, Raphanus raphanistrum* and *Rumex crispus.*

Conditions: spraying commenced at 12:30 pm, the alluvial soil was dry, cloud cover was <10%, temperature ~ 17 °C, and the wind was easterly at ~ 5 knots.

| | | |
|---|---|---|
| Wesley Vale: | *Papaver somniferum* | 103 |
| | *Fumaria muralis* | 17 |
| | *Chenopodium album* | 26 |

Other species 30, including *Polygonum aviculare, Polygonum convolvulus, Trifolium repens* and *Lamium amplexicaule.*

Conditions: spraying commenced at 11:00 am, the krasnozem soil was dry, cloud cover was 20-25%, temperature ~ 12 °C, and the wind was westerly at ~ 10 knots.

### 8.2.1 Efficacy

Following the post emergent application of the diquat treatments the plants were grown for one week before analysis. Nine different levels of damage were defined and graded from 1 to 9 as described below:

| Rating | Plant Description |
|---|---|
| 1 | Complete plant kill |
| 2 | Heavy damage to complete kill |
| 3 | Severe damage |
| 4 | Recognisable burning and yellowing of leaves |
| 5 | Limit of commercial acceptability |
| 6 | More severe symptoms |
| 7 | Mild but clearly recognisable symptoms |
| 8 | Very mild symptoms |
| 9 | Healthy plant |

After scoring the plants in each treatment ten plants were cut at soil level, labelled, placed in sealed plastic bags and returned to the University at Hobart. Here the fresh weights of the plants was measured. This value was expressed as a percentage of the control.

### 8.2.2 Spray retention

**Part a:** The objective of this experiment was to determine if the EC performed similarly to diclofop-methyl, in regards to spray retention when in combination with diquat. This was conducted only at the Swansea site.

After application of the diquat treatments three plants of poppy, fat hen and fumitory were harvested from the diquat + diclofop-methyl and the diquat + EC plots from the ethofumesate/asulam treatment. These plants were carefully placed in polystyrene containers and taken back to the University at Hobart. Here spray retention on the plants was observed under ultra-violet light in a dark cabinet. Fluorescent spray deposits on leaf surfaces were photographed using a Pentax 35mm SLR camera fitted with a Hoya photographic filter (type yellow K2). This filter reduces the transmittance of wavelengths below 550 mμ (ie in the UV range) to zero, enabling distinctive recordings of spray retention to be made.

**Part b:** At the first trial the water soluble dye, rhodamine b was added at a concentration of 0.1%. After the dye had dried on the leaves, five plants of poppy, fat hen and fumitory were harvested at soil level and washed in known volumes of water (5-100 ml depending on plant size) to redissolve the dye. On inspection of the wash solutions it was revealed that concentration of dye used was too low. Hence, the wash solutions were discarded and at the Wesley Vale trial the concentration of dye was increased to 2%.

After washing the plants as outlined above, the plants were labelled and placed in a sealed plastic bag and taken back to the laboratories of Tasmanian Alkaloids. Here a subsample of three plants was removed from the bag and leaf areas measured using a planimeter. On inspection of the plants it was revealed that the water had not removed all the dye from the leaf surface. The dye appeared to be absorbed through the leaf cuticle. To remove this dye the plants were washed again in 25mls of 5% acetic acid. The dye concentrations in both of the 'washing' solutions was then estimated using a

Sequoia-Turner, (model 340) spectrophotometer at a wavelength of 550 m$\mu$. After which the amount of herbicide retained ($\mu$l) per leaf surface area (cm$^2$) was calculated and expressed as a percentage of the control.

## 8.3 Results and Discussion

It was not the intention of this experiment to compare responses between sites, and for this reason the results from each trial will be examined independently.

### 8.3.1 Swansea

The site conditions at the time of spraying are shown in Plate 10.0. One week after application of the herbicide treatments, it was observed that there was no distinct difference between plant response to diquat combined with diclofop-methyl and diquat combined with the EC. There was no apparent influence of ethofumesate/asulam on plant response. Both these observations are illustrated in Plate 11.0.

#### 8.3.1.a Spray retention

The objective of this experiment was to determine if the EC performed similarly to diclofop-methyl, in regards to spray retention when in combination with diquat. It was also possible to examine herbicide retention on the leaves of those plants examined in the previous experiments. The results are illustrated in Plates 12.0 to 14.0.

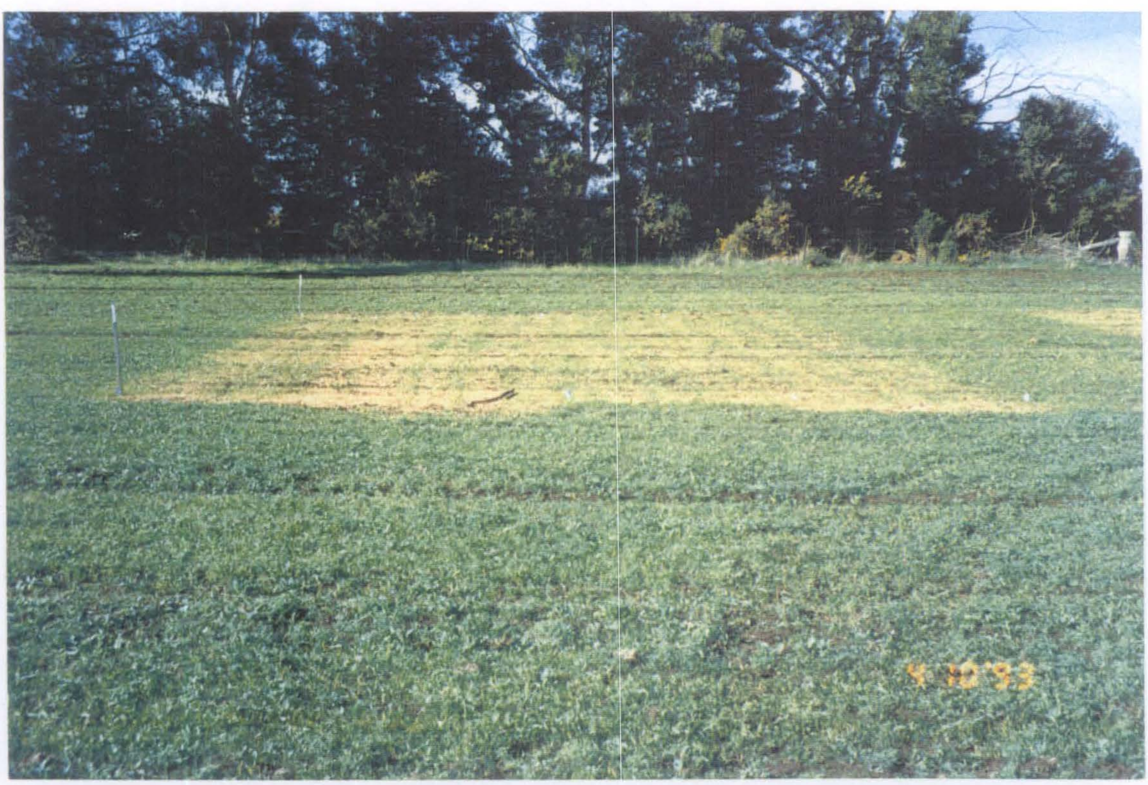
On each plant species examined there were no distinctive differences between the retention of the diquat + diclofop-methyl, compared with the diquat + EC herbicide treatments. This, in association with the observations from Plate 11.0, provides support for the hypothesis that both diclofop-methyl and EC perform similarly when in admixture with diquat.

Plate 10.0

Swansea field trial.
Application of diquat treatments.


Plate 11.0

Comparison of diclofop-methyl and EC.



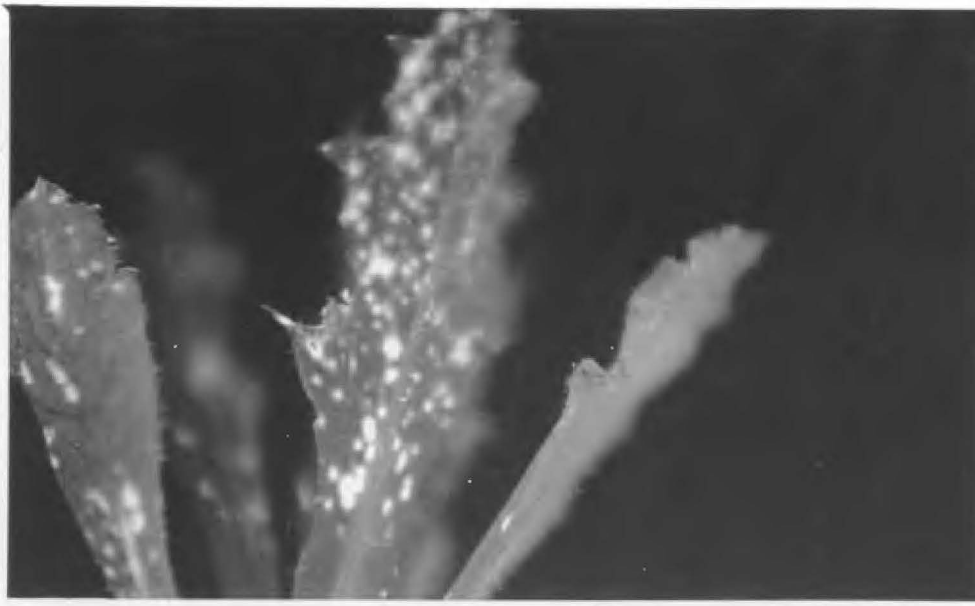

**diclofop-methyl** **EC**

+: ethofumesate/asulam

-: water

Plate 12.0

Distribution of diquat on poppy leaves.



diquat + diclofop-methyl

diquat + EC

Plate 13.0

Distribution of diquat on fumitory leaves.

diquat + diclofop-methyl

diquat + EC

Plate 14.0

Distribution of diquat on fat hen leaves.

diquat + diclofop-methyl

diquat + EC

There were no observable differences between retention on young or old leaves on any of the plants, however, it was noted from Plate 12.0 that a considerable amount of herbicide was retained on the abaxial surface of poppy leaves. Measurements of leaf wettability in the earlier experiments only considered the adaxial surface. However, Seamen (1979) has reported that lower contact angles exist on the abaxial surface compared with the adaxial surface on the leaves of a number of weed species. This would explain the greater retention observed on the abaxial surface of poppy leaves, and depending on leaf orientation at the time of spraying would influence the level of herbicide damage.

### 8.3.1.b Spray efficacy

The efficacy of the herbicide treatments, determined by scoring plant responses, are summarised in Table 8.1 (Appendix 5).

**Table 8.1** Herbicide efficacy. (scored 1-9; dead>healthy)

| | Poppy | | Fumitory | | Fat Hen | |
|---|---|---|---|---|---|---|
| Treatment | + | - | + | - | + | - |
| water | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 | 9.00 |
| diquat | 8.00 | 8.00 | 7.67 | 8.00 | 7.67 | 8.00 |
| diq. + d/methyl | 7.00 | 7.00 | 5.33 | 6.00 | 5.33 | 4.67 |
| diq. + EC | 6.67 | 6.33 | 5.33 | 5.33 | 5.67 | 5.33 |
| diq. + New form. | 6.67 | 6.33 | 5.67 | 5.33 | 5.33 | 5.00 |
| diq. + agral | 6.33 | 6.67 | 5.33 | 5.67 | 6.33 | 6.00 |

lsd=0.70 (t=0.05)

+ ethofumesate/asulam
- water

The addition of a surfactant to diquat significantly decreased the mean scores on all plants, supporting the findings made in earlier experiments that surfactants enhance wetting of leaf surfaces. The weeds tended to score lower (ie greater damage), compared with poppy under all herbicide treatments, and at no time does a significant difference exist between the response achieved with diclofop-methyl compared with either the EC or the New formulation.

Two important points to consider are the fact that the ethofumesate/asulam treatment had no effect on weed or crop plants, and that Agral®, which performed similarly to diclofop-methyl, did not induce the expected severe burning of crop and weed plants. These observations are common throughout this experiment, and an attempt to explain the findings will be made in the discussion of the Wesley Vale trial.

Table 8.2 (Appendix 5), presents a comparison of fresh weights. This data indicates the effects of the herbicide treatments. It is clear from this table that diquat, without surfactant, does not significantly reduce plant weights compared with the control.

**Table 8.2** Plant fresh weights expressed as a percentage of the control.

| | Poppy | | Fumitory | | Fat Hen | |
|---|---|---|---|---|---|---|
| Treatment | + | - | + | - | + | - |
| diquat | 100.82 | 91.56 | 80.70 | 98.55 | 92.60 | 88.36 |
| diq. + d/methyl | 86.59 | 83.06 | 74.14 | 55.90 | 84.51 | 77.05 |
| diq. + EC | 84.43 | 74.26 | 78.29 | 63.20 | 76.53 | 73.51 |
| diq. + New form. | 83.41 | 81.41 | 77.72 | 66.67 | 83.64 | 73.77 |
| diq. + agral | 66.43 | 65.56 | 69.19 | 53.38 | 82.49 | 76.57 |

**lsd=14.50 (t=0.05)**

+ ethofumesate/asulam
- water

For both poppy and fumitory there was a tendency for the Agral® treatment to reduce plant weights. However, observations in Table 8.1, are consistent with findings in Table 8.2.

From this trial, the hypothesis proposed in the introduction of this experiment, that the EC of the commercial formulation of diclofop-methyl creates the synergistic relationship with diquat, has been substantiated. By conducting a second trial, at Wesley Vale, it was aimed to validate this finding through determination of efficacy and retention of the herbicide treatments.

### 8.3.2 Wesley Vale

The site conditions at the date of spraying are illustrated in Plate 15.0. One week after application of the herbicide treatments, a comparison between the diquat + EC and the control (ie water) treatments was made (Plate 16.0). Initially from this photograph it appears that the control treatment produces the best results. However, on closer inspection it was apparent that there remained a dense coverage of weeds in the control plot which in time would reduce yields through competition, and also contaminate and interfere with harvesting of the final product. The discussion of spray efficacy will examine this observation in more detail.

#### 8.3.2.a Spray efficacy

The efficacy of the herbicide treatments, determined by scoring plant responses, are summarised in Table 8.3 (Appendix 5).

**Table 8.3** Herbicide efficacy. (scored 1-9; dead > healthy)

| | Poppy | | Fumitory | | Fat Hen | |
|---|---|---|---|---|---|---|
| Treatment | + | - | + | - | + | - |
| water | 9.00 | 9.00 | 8.00 | 9.00 | 8.00 | 9.00 |
| diquat | 8.00 | 9.00 | 4.67 | 6.00 | 5.67 | 6.00 |
| diq. + d/methyl | 7.33 | 7.67 | 3.33 | 5.33 | 3.67 | 6.00 |
| diq. + EC | 7.00 | 7.67 | 3.67 | 5.33 | 4.33 | 5.33 |
| diq. + New form. | 7.00 | 7.67 | 3.67 | 5.00 | 4.00 | 5.00 |
| diq. + agral | 7.67 | 8.00 | 4.67 | 5.67 | 4.00 | 5.00 |

**lsd=1.15 (t=0.05)**

+ ethofumesate/asulam
- water

For all herbicide treatments there was a pronounced difference between the response of poppy compared with fumitory and fat hen. There was also a noticeable effect of the ethofumesate/asulam treatment on subsequent diquat treatments for the weed species. It was interesting to note that there was no significant effect of adding a surfactant to diquat on the plants examined, however, there was a significant difference between the response of the weed species treated with diquat compared with the control. Therefore, it is possible that the epicuticular waxes, identified in the previous experiment, of the

## Plate 15.0

**Wesley Vale field trial.**
**Site conditions.**

## Plate 16.0

**Comparison of diquat + EC and control (water).**

**diquat + EC** **control (water)**

weeds are inhibited by the environmental conditions while the waxes of poppy leaves are not affected. As demonstrated from the above results the use of diquat alone would have achieved the same results as if a surfactant had been included in the spray formulation.

Table 8.4 also examines the efficacy of herbicide treatments. There was a greater reduction in fresh weights of fumitory and fat hen plants compared with poppy plants, however, there was no significant effect of the ethofumesate/asulam treatment on the plants.

**Table 8.4.** Plant fresh weights expressed as a percentage of the control.

| | Poppy | | Fumitory | | Fat Hen | |
|---|---|---|---|---|---|---|
| Treatment | + | - | + | - | + | - |
| diquat | 74.71 | 71.57 | 20.64 | 29.62 | 35.42 | 16.71 |
| diq. + d/methyl | 60.38 | 62.72 | 13.41 | 21.50 | 20.62 | 14.94 |
| diq. + EC | 61.74 | 60.16 | 20.35 | 30.28 | 23.66 | 13.05 |
| diq. + New form. | 47.71 | 67.01 | 21.62 | 22.99 | 27.34 | 12.74 |
| diq. + agral | 59.22 | 55.21 | 22.91 | 23.04 | 22.56 | 11.92 |

**lsd=16.50 (t=0.05)**

+ ethofumesate/asulam
- water

The efficacy of a number of the diquat treatments, applied after the ethofumesate/asulam treatments, is illustrated in Plates 17.0 to 20.0. These figures were obtained by leaving a marker in the ground at the point where the first photograph was taken. Plate 17.0 supports the findings that diquat, with out the addition of a surfactant, is not capable of controlling 'hard' to wet weed species. One week from application of the herbicide treatment, the leaf margins of crop and weed plants were desiccated, but, the majority of plants present in the second photograph will recover from the spray application.

Plates 18.0 and 19.0 illustrate the effects of diquat + diclofop-methyl and diquat + EC respectively. It is apparent that fat hen and fumitory have been adequately controlled by each treatment. Although the poppy plants were desiccated by the herbicide

**Plate 17.0**

**Response of crop and weed species to diquat.**


*immediately after spraying*


*one week after spraying*

**Plate 18.0**
**Response of crop and weed species to diquat + diclofop-methyl.**


*immediately after spraying*


*one week after spraying*

**Plate 19.0**
**Response of crop and weed species to diquat + EC.**


*immediately after spraying*


*one week after spraying*

**Plate 20.0**
**Response of crop and weed species to diquat + agral.**


*immediately after spraying*


*one week after spraying*

treatments, these plants have the potential to recover quickly and develop into healthy plants, inhibiting the progress of those weed species still present, and any weeds that appear after the diquat + surfactant treatment.

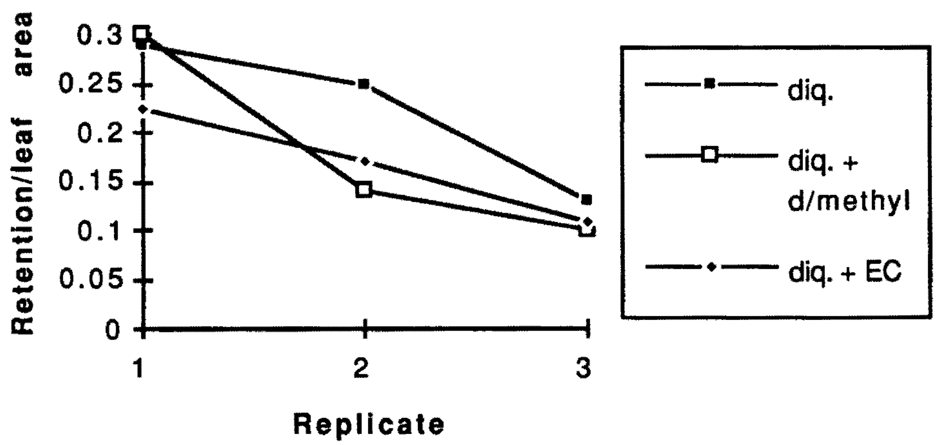
Plate 20.0 examines the efficacy of the diquat + Agral® treatment. This treatment was very effective in controlling fumitory and fat hen plants, and had no apparent detrimental effect on poppy plants. From this observation it is possible to support the future use of this surfactant in admixture with diquat. However, the use of Agral® has previously destroyed poppy plants, and it appears that the efficacy of this treatment is strongly influenced by environmental conditions.

### 8.3.2.b <u>Spray retention</u>

The aim of this experiment was to correlate the retention of the herbicide treatments with spray efficacy for each plant. Unfortunately, due to problems associated with the developed technique no relation could be achieved.

When washing the plants at the site, it was observed that the intensity of dye in the wash solutions decreased with time from spraying the diquat treatments. This trend is illustrated in Figure 8.1. A similar response was observed with fumitory and fat hen

**Figure 8.1** Decrease in spray retention with time for poppy plants.




(nb replicate 1 corresponds to the first washing)

This graph tends to suggest that diquat, without surfactant, resulted in the greatest level of herbicide retention. However, on inspection of Plates 17.0 to 20.0 it is obvious that the addition of diclofop-methyl or EC to diquat resulted in greater spray retention, compared with diquat, on poppy plants. The dye on the plants from the later washings could not be removed with water, suggesting that the addition of a surfactant enhances the penetration of diquat through the leaf cuticle. This would be expected to increase herbicide efficacy, however, Bland and Brian (1975, cited in Seamen, 1979) have reported that although surfactants can enhance uptake of foliar applied herbicides, they may in fact inhibit their movement within the plant.

Attempts were made to remove the absorbed dye by washing the harvested plants in acetic acid. The acetic acid was not able to remove all the remaining dye, therefore, a correlation with spray efficacy was not possible. If spray retention is to be measured in future trial work, it would be advisable to wash the plants immediately after spraying with the herbicide treatments. This would ensure that the time from spraying to washing would not influence results.

It was demonstrated in earlier experiments that epicuticular wax development is important in controlling the response of plant species to foliar applied herbicides. The deposition of these waxes is modified by herbicidal and environmental factors, and, if this deposition could be monitored in the field it would enable growers to anticipate the level of crop/weed damage from a given herbicide treatment. Such a test exists for use in peas (Fryer and Makepeace, 1978), and it would be possible to develop the dye used in this study for such a test.

During the course of the trial two important observations have been made, that went against all that was established in the laboratory. The first was the apparent lack of, or unpredictable, response of plant species to the ethofumesate/asulam treatment. The second, was the inability of Agral®, when formulated with diquat, to reduce the margin of herbicide selectivity that exists between weed and crop species, resulting in poppy and weed kill. Environment factors can readily alter the nature of the leaf surface, and it could be argued that this contributed to the first observation. However, problems associated with experimental techniques could also be responsible. For instance, when scoring/weighing plants it is difficult to remain subjective, as there is a tendency to only notice those plants that are visible (ie alive). In future trial work, consideration should be given to the period of time between, applying the diquat treatments and scoring/weighing plants. If time permitted a comparison of yields from herbicide treatments, in relation to a hand weeded control, would, in conjunction with the present measurements be worthwhile.

A possible explanation for the poor effect of Agral® (Plate 20.0), is that the plots were sprayed with the diquat treatments in the morning, which is in contrast to the commercially recommended spraying time of late afternoon. The late afternoon treatment enables some internal transport of diquat during the night, before acute phytotoxicity is induced the following day by light. The interconversion of the diquat ion to the free radical form limits further internal transport (Klingman and Ashton, 1982), therefore, although Agral® may have increased leaf wettability, the observed plant responses were in part due to the time of spraying. The time of spraying would also be expected to increase the effectiveness of the other diquat treatments, increasing the degree of crop and weed damage. This assumption was examined at the Wesley Vale trial where the commercial crop was sprayed on the same day, using the standard herbicide treatment, at 7:00 pm. Comparison of the crop with the equivalent trial plot the following week, revealed that a far better degree of weed control, with no increased crop damage was achieved. As there was no change in crop response to the time of spraying, it is possible that the translocation and metabolism of the diquat ion plays an important role in selectivity.

The examination of spray retention and efficacy has demonstrated that no significant difference exists between the response of plant species to diclofop-methyl, EC or New formulation in admixture with diquat. This provides support for future field trial work with the EC aimed at replacing diclofop-methyl. It also dismisses any concerns in regards to the viability of the New diclofop-methyl formulation.

# 9. General Discussion

# 9 GENERAL DISCUSSION

A glasshouse and field trial was undertaken to determine the mechanism of herbicide selectivity in poppy crops. In establishing this mechanism, it was possible to account for the narrow margin of herbicide selectivity which exists with the current herbicide program.

A laboratory technique developed for the measurement of leaf wettability, demonstrated that the leaves of poppy and a number of weed species are 'hard' to wet, and diclofop-methyl has properties characteristic of a surfactant when in admixture with diquat (ie can lower contact angles of diquat solutions). It was established that the use of a powerful surfactant, able to reduce the contact angles of diquat on crop and weed species, (eg Agral®), would potentially decrease herbicide selectivity.

Measurements of dynamic surface tension supported the findings that Agral® is a strong surfactant in comparison with diclofop-methyl. Therefore, a surfactant which does not have a dramatic effect on contact angles, or dynamic surface tension, is required for incorporation with diquat. These observations can be used to screen possible replacements for diclofop-methyl.

Although the incorporation of a surfactant, such as diclofop-methyl, to diquat would increase the wetting of those plants categorised as 'hard' to wet, it would not account for any selectivity achieved between crop and weed species with this treatment. As the measurement of contact angles on leaf surfaces represents a surface phenomenon, consideration was given to the possibility that the ethofumesate/asulam treatment, which precedes the diquat/diclofop-methyl treatment, could increase herbicide selectivity by influencing the surface fine structure of leaves.

Contact angle measurements made after ethofumesate/asulam treatments, using diquat solutions, supported this proposal, and identified ethofumesate as the instigator of this change. Examination of leaf epicuticular waxes revealed that ethofumesate selectively inhibits wax deposition on the developing leaves of a number of weed species, yet, the epicuticular waxes of poppy leaves are not susceptible to inhibition by ethofumesate. Inhibition of wax deposition on developing weed leaves, will effectively increase the retention of foliar applied herbicides, which would account for the selectivity achieved with the current herbicide program.

Field trials were conducted in the east and north-west of Tasmania to assess the laboratory findings. As diclofop-methyl behaves as a surfactant when in admixture with diquat, it was proposed that it was the inert ingredients (eg organic solvents and adjuvants), and not the active ingredient (ie diclofop-methyl), that decreased contact angle and surface tensions of diquat solutions, determined in the laboratory. Therefore, the trials examined the potential of these inert ingredients to act as a replacement for diclofop-methyl.

Through measurements of spray retention and efficacy it was demonstrated that environmental factors have a marked influence on the effect of foliar applied diquat treatments. Adequate weed control was possible at Wesley Vale without the addition of a surfactant, and such a result would clearly be dependent on environmental influences. This situation could be taken advantage of if the development of epicuticular waxes could be monitored by growers. The dye, (rhodamine b), used in the spray retention analysis could be developed for this purpose.

It was observed that ethofumesate treatments infrequently influenced plant responses to diquat treatments. These results demonstrate that limitations do exist in extrapolating results made from plants grown under glasshouse conditions to field conditions.

Examination of spray retention and efficacy at both sites clearly demonstrated that the inert ingredients of the commercial formulation of diclofop-methyl, would, in admixture with diquat produce the same result compared with the current diquat/diclofop-methyl treatment. Although this would not increase herbicide selectivity, it would reduce the volume of herbicides required to achieve an adequate level of weed control in poppy crops.

In conclusion, the nature of the leaf surface of poppy and weed plants, has a pronounced effect on the efficacy of the diquat/diclofop-methyl treatment. If these surfaces are influenced by environmental conditions and/or ethofumesate applications, herbicide selectivity can be modified. This points to the fact that a narrow margin of herbicide selectivity currently exists in poppy crops, which could, under unfavourable circumstances, result in excessive crop damage or insufficient weed control. Clearly this is an undesirable situation, and if not overcome has the potential to limit the future development of the poppy industry in Tasmania. It is hoped the information made available through this work will help address this problem.

# 10. References

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# 11. Appendices

# Appendix 1

## Poppy

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 138 | 142 | 138 | 144 | 143 | 147 | 142 |
| diquat | 133 | 137 | 139 | 140 | 131 | 141 | 137 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 132 | 134 | 130 | 134 | 136 | 136 | 134 |
| 0.01 | 126 | 128 | 136 | 136 | 136 | 131 | 132 |
| 0.10 | 109 | 108 | 106 | 114 | 114 | 111 | 110 |
| 1.00 | 63 | 66 | 62 | 65 | 63 | 65 | 64 |
| 10.00 | 34 | 38 | 38 | 43 | 38 | 38 | 38 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 126 | 132 | 129 | 128 | 127 | 132 | 129 |
| 0.01 | 112 | 110 | 110 | 109 | 111 | 108 | 110 |
| 0.10 | 47 | 49 | 51 | 52 | 51 | 46 | 49 |
| 1.00 | 41 | 46 | 41 | 46 | 45 | 39 | 43 |
| 10.00 | 36 | 37 | 39 | 41 | 36 | 34 | 37 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 130 | 131 | 136 | 134 | 138 | 139 | 135 |
| 0.01 | 130 | 138 | 129 | 131 | 131 | 125 | 131 |
| 0.10 | 117 | 120 | 118 | 122 | 112 | 116 | 118 |
| 1.00 | 110 | 104 | 109 | 106 | 104 | 109 | 107 |
| 10.00 | 109 | 104 | 108 | 105 | 103 | 104 | 106 |

## P.rhoeas

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 123 | 124 | 129 | 124 | 131 | 123 | 126 |
| diquat | 132 | 129 | 126 | 136 | 137 | 136 | 133 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 119 | 120 | 124 | 116 | 125 | 121 | 121 |
| 0.01 | 113 | 110 | 107 | 114 | 113 | 109 | 111 |
| 0.10 | 98 | 99 | 99 | 101 | 102 | 105 | 101 |
| 1.00 | 56 | 49 | 59 | 54 | 54 | 54 | 54 |
| 10.00 | 30 | 32 | 34 | 36 | 33 | 31 | 33 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 126 | 130 | 124 | 129 | 128 | 125 | 127 |
| 0.01 | 115 | 109 | 103 | 115 | 96 | 105 | 107 |
| 0.10 | 44 | 45 | 35 | 39 | 44 | 42 | 42 |
| 1.00 | 35 | 40 | 32 | 37 | 37 | 45 | 38 |
| 10.00 | 39 | 40 | 35 | 36 | 38 | 39 | 38 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 125 | 126 | 126 | 131 | 124 | 131 | 127 |
| 0.01 | 120 | 125 | 125 | 120 | 122 | 119 | 122 |
| 0.10 | 111 | 112 | 109 | 111 | 108 | 109 | 110 |
| 1.00 | 110 | 106 | 104 | 106 | 103 | 104 | 106 |
| 10.00 | 101 | 102 | 101 | 104 | 101 | 98 | 101 |

## Fat Hen

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 144 | 138 | 141 | 135 | 135 | 138 | 139 |
| diquat | 133 | 136 | 136 | 131 | 132 | 131 | 133 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 123 | 133 | 131 | 125 | 132 | 130 | 129 |
| 0.01 | 122 | 133 | 125 | 139 | 126 | 128 | 129 |
| 0.10 | 104 | 103 | 109 | 104 | 103 | 100 | 104 |
| 1.00 | 63 | 59 | 58 | 56 | 50 | 52 | 56 |
| 10.00 | 29 | 32 | 36 | 33 | 33 | 27 | 32 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 124 | 128 | 127 | 124 | 124 | 126 | 126 |
| 0.01 | 103 | 103 | 101 | 103 | 101 | 104 | 103 |
| 0.10 | 44 | 45 | 44 | 46 | 43 | 44 | 44 |
| 1.00 | 46 | 43 | 44 | 45 | 40 | 44 | 44 |
| 10.00 | 38 | 42 | 42 | 37 | 44 | 37 | 40 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 138 | 131 | 138 | 131 | 135 | 138 | 135 |
| 0.01 | 128 | 133 | 129 | 132 | 127 | 126 | 129 |
| 0.10 | 120 | 123 | 117 | 116 | 117 | 114 | 118 |
| 1.00 | 100 | 97 | 104 | 103 | 107 | 109 | 103 |
| 10.00 | 96 | 95 | 94 | 101 | 91 | 96 | 96 |

## Fumitory

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 125 | 127 | 124 | 122 | 127 | 125 | 125 |
| diquat | 122 | 121 | 118 | 119 | 123 | 117 | 120 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 119 | 118 | 116 | 121 | 120 | 123 | 120 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.01 | 116 | 116 | 118 | 119 | 115 | 116 | 117 |
| 0.10 | 104 | 110 | 111 | 106 | 104 | 105 | 107 |
| 1.00 | 55 | 54 | 54 | 56 | 53 | 49 | 54 |
| 10.00 | 34 | 34 | 33 | 35 | 32 | 33 | 34 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 117 | 117 | 121 | 117 | 119 | 118 | 118 |
| 0.01 | 109 | 106 | 105 | 107 | 105 | 110 | 107 |
| 0.10 | 42 | 41 | 46 | 44 | 43 | 39 | 43 |
| 1.00 | 42 | 41 | 39 | 40 | 41 | 39 | 40 |
| 10.00 | 37 | 39 | 42 | 38 | 36 | 38 | 38 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 121 | 116 | 117 | 121 | 115 | 118 | 118 |
| 0.01 | 114 | 116 | 118 | 116 | 114 | 116 | 116 |
| 0.10 | 109 | 109 | 109 | 110 | 112 | 111 | 110 |
| 1.00 | 101 | 102 | 105 | 107 | 105 | 103 | 104 |
| 10.00 | 103 | 101 | 103 | 100 | 104 | 101 | 102 |

## Wild radish

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 104 | 103 | 101 | 98 | 97 | 102 | 101 |
| diquat | 94 | 92 | 92 | 93 | 96 | 88 | 93 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 76 | 72 | 75 | 78 | 76 | 71 | 75 |
| 0.01 | 68 | 64 | 64 | 68 | 63 | 62 | 65 |
| 0.10 | 57 | 57 | 53 | 59 | 53 | 52 | 55 |
| 1.00 | 43 | 41 | 39 | 43 | 38 | 44 | 41 |
| 10.00 | 31 | 31 | 32 | 34 | 34 | 40 | 34 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 84 | 86 | 89 | 81 | 87 | 87 | 86 |
| 0.01 | 62 | 60 | 56 | 62 | 60 | 65 | 61 |
| 0.10 | 40 | 41 | 35 | 41 | 42 | 40 | 40 |
| 1.00 | 36 | 40 | 35 | 38 | 35 | 39 | 37 |
| 10.00 | 35 | 37 | 36 | 37 | 38 | 35 | 36 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 86 | 88 | 86 | 90 | 92 | 96 | 90 |
| 0.01 | 80 | 83 | 85 | 90 | 80 | 87 | 84 |
| 0.10 | 68 | 66 | 66 | 64 | 63 | 68 | 66 |
| 1.00 | 60 | 55 | 57 | 56 | 64 | 55 | 58 |
| 10.00 | 55 | 54 | 55 | 56 | 54 | 55 | 55 |

## Shep. purse

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 88 | 92 | 94 | 96 | 94 | 89 | 92 |
| diquat | 104 | 102 | 96 | 98 | 99 | 93 | 99 |
| Hoe (% v/v): | | | | | | | |
| 1.00E-03 | 84 | 76 | 75 | 81 | 81 | 76 | 79 |
| 0.01 | 52 | 50 | 54 | 51 | 49 | 55 | 52 |
| 0.10 | 52 | 44 | 48 | 50 | 50 | 51 | 49 |
| 1.00 | 44 | 43 | 42 | 39 | 41 | 46 | 43 |
| 10.00 | 41 | 40 | 43 | 39 | 38 | 36 | 40 |
| Agral (% v/v) | | | | | | | |
| 1.00E-03 | 92 | 89 | 79 | 85 | 86 | 95 | 88 |
| 0.01 | 63 | 62 | 60 | 57 | 54 | 56 | 59 |
| 0.10 | 37 | 41 | 44 | 43 | 44 | 42 | 42 |
| 1.00 | 40 | 41 | 43 | 40 | 37 | 39 | 40 |
| 10.00 | 37 | 41 | 38 | 41 | 40 | 37 | 39 |
| NK (% v/v) | | | | | | | |
| 1.00E-03 | 89 | 90 | 89 | 84 | 89 | 91 | 89 |
| 0.01 | 88 | 90 | 87 | 84 | 87 | 82 | 86 |
| 0.10 | 64 | 65 | 57 | 62 | 64 | 59 | 62 |
| 1.00 | 54 | 55 | 58 | 63 | 63 | 63 | 59 |
| 10.00 | 51 | 54 | 59 | 54 | 53 | 54 | 54 |

## Spear thistle

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 48 | 55 | 49 | 47 | 47 | 47 | 49 |
| diquat | 57 | 50 | 47 | 49 | 46 | 52 | 50 |

## Curled dock

| treatment | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| water | 37 | 38 | 29 | 31 | 31 | 34 | 33 |
| diquat | 29 | 25 | 36 | 32 | 37 | 29 | 31 |

General Linear Models Procedure

Dependent Variable: ANGle

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 112 | 815072.1543 | 7277.4299 | 783.29 | 0.0001 |
| Error | 535 | 4970.5849 | 9.2908 | | |
| Corrected Total | 647 | 820042.7392 | | | |

| R-Square | C.V. | Root MSE | ANG Mean |
|---|---|---|---|
| 0.993939 | 3.504352 | 3.048083 | 86.97994 |

General Linear Models Procedure

Dependent Variable: ANGle x

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 5 | 83.5818 | 16.7164 | 1.80 | 0.1112 |
| PLT | 5 | 163817.2299 | 32763.4460 | 3526.43 | 0.0001 |
| CONC | 5 | 415656.2855 | 83131.2571 | 8947.68 | 0.0001 |
| SURF | 2 | 94038.2809 | 47019.1404 | 5060.82 | 0.0001 |
| PLT*CONC | 25 | 23633.2793 | 945.3312 | 101.75 | 0.0001 |
| PLT*SURF | 10 | 12284.3673 | 1228.4367 | 132.22 | 0.0001 |
| CONC*SURF | 10 | 79271.7006 | 7927.1701 | 853.23 | 0.0001 |
| PLT*CONC*SURF | 50 | 26287.4290 | 525.7486 | 56.59 | 0.0001 |

# Appendix 2

## Diclofop-methyl + 5% diquat
(% v/v)

| % (v/v) | 0.01 | | 0.032 | | 0.1 | |
|---|---|---|---|---|---|---|
| | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) |
| | 2.6 | 74.2 | 3 | 73.7 | 2.8 | 70.7 |
| | 5.8 | 74 | 5.44 | 73.9 | 5.3 | 71.6 |
| | 10.7 | 73.6 | 10.9 | 73.4 | 11.1 | 71.7 |
| | 15.5 | 73.5 | 15.8 | 73.3 | 15.6 | 71.6 |
| | 20.4 | 73.3 | 19.7 | 72.9 | 21.2 | 71.4 |
| | 25.7 | 72.9 | 25.6 | 72.7 | 26.2 | 71.1 |
| | 30.2 | 72.4 | 31.6 | 71.6 | 31.9 | 69.5 |

## Agral + 5% diquat
(% v/v)

| % (v/v) | 0.01 | | 0.032 | | 0.1 | |
|---|---|---|---|---|---|---|
| | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) |
| | 3 | 71 | 3.2 | 64.9 | 3.73 | 52.4 |
| | 5.8 | 72.3 | 5.39 | 67.9 | 5.87 | 56.8 |
| | 10.9 | 73 | 10.4 | 70.1 | 10.5 | 62.6 |
| | 15.8 | 72.8 | 15.8 | 70.5 | 16.2 | 65.7 |
| | 20.2 | 72.6 | 20.5 | 70.9 | 21.2 | 66.9 |
| | 26 | 72.5 | 25.7 | 70.9 | 25.4 | 67.2 |
| | 31 | 71.4 | 31 | 69.5 | 29.7 | 65.5 |

## Newkalgen + 5% diquat
(% v/v)

| % (v/v) | 0.01 | | 0.032 | | 0.1 | |
|---|---|---|---|---|---|---|
| | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) | freq (hz) | d.s.t (dynes/cm) |
| | 2.7 | 72.5 | 3 | 68.5 | 2.7 | 59.2 |
| | 5.2 | 72.6 | 5.5 | 70.2 | 6.3 | 58.2 |
| | 11 | 72.6 | 11.3 | 70.3 | 10.1 | 64.7 |
| | 15.8 | 72.5 | 15.7 | 70.3 | 16.2 | 66.2 |
| | 20.4 | 72.4 | 21.5 | 70.2 | 20.9 | 66.8 |
| | 25.4 | 72.1 | 25.9 | 70.2 | 25.8 | 66.9 |
| | 31 | 70.6 | 30.7 | 68.7 | 31.2 | 66.4 |

## Diquat (0.5% v/v)

| freq (hz) | d.s.t (dynes/cm) |
|---|---|
| 3.2 | 73.3 |
| 5.1 | 73.5 |
| 10.4 | 73.3 |
| 16 | 72.9 |
| 20.5 | 72.6 |
| 25.8 | 72.3 |
| 31 | 71.8 |

# THE MEASUREMENT OF DYNAMIC SURFACE TENSION

The surface tension of a liquid can be measured as follows:

* Bubbles of air are blown through a fine capillary of known radius into the test solution.

* The pressure drop across the bubble interface is detected and measured.

* Surface Tension can be calculated by transposition of the Laplace Equation.

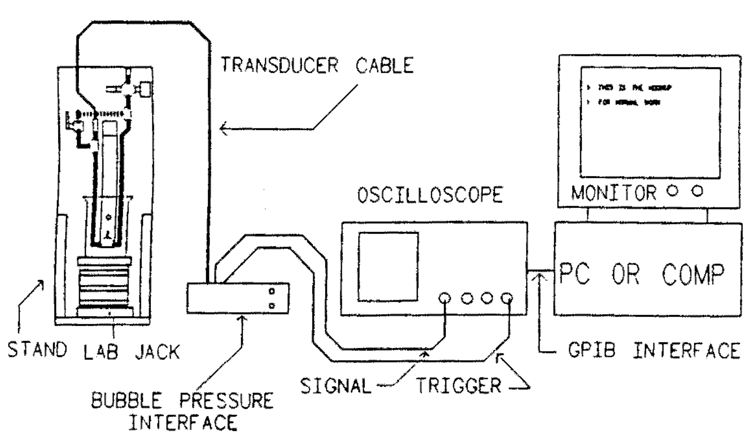
$$\Delta P = \frac{2 \times \gamma}{r}$$

Where: $\Delta P$ = Pressure drop across the bubble interface

$r$ = Radius of the bubble

$\gamma$ = Surface tension of the solution

The Dynamic Surface Tension can be measured by varying the frequency of bubble formation. The faster bubbles are generated the less time surfactant has to migrate to the bubble interface and reduce the surface tension. The Dynamic Surface Tension for the test solution can be represented by plotting surface tension vs frequency or surface tension vs bubble period. A schematic diagram of our equipment is demonstrated in Figure 1.



# Appendix 3

## Poppy

| Young leaf | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| ***water*** | | | | | | | |
| control | 134 | 133 | 139 | 137 | 136 | 138 | 136 |
| asulox | 129 | 130 | 131 | 134 | 135 | 136 | 133 |
| tramat | 137 | 133 | 134 | 130 | 137 | 130 | 134 |
| tramat/asulox | 130 | 132 | 133 | 130 | 131 | 132 | 131 |
| ***diquat*** | | | | | | | |
| control | 140 | 138 | 134 | 133 | 133 | 134 | 135 |
| asulox | 135 | 134 | 132 | 136 | 130 | 134 | 134 |
| tramat | 130 | 135 | 130 | 134 | 128 | 128 | 131 |
| tramat/asulox | 134 | 131 | 131 | 130 | 129 | 131 | 131 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 62 | 63 | 59 | 60 | 61 | 61 | 61 |
| asulox | 54 | 58 | 60 | 54 | 52 | 55 | 56 |
| tramat | 60 | 62 | 60 | 59 | 64 | 61 | 61 |
| tramat/asulox | 56 | 60 | 59 | 64 | 62 | 58 | 60 |

| old leaf | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| ***water*** | | | | | | | |
| control | 144 | 142 | 139 | 145 | 142 | 141 | 142 |
| asulox | 134 | 132 | 124 | 125 | 124 | 129 | 128 |
| tramat | 125 | 128 | 126 | 123 | 121 | 124 | 125 |
| tramat/asulox | 112 | 110 | 116 | 118 | 114 | 112 | 114 |
| ***diquat*** | | | | | | | |
| control | 138 | 134 | 134 | 134 | 136 | 137 | 136 |
| asulox | 130 | 124 | 126 | 131 | 127 | 125 | 127 |
| tramat | 118 | 116 | 120 | 119 | 112 | 114 | 117 |
| tramat/asulox | 109 | 111 | 112 | 114 | 112 | 109 | 111 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 69 | 66 | 64 | 62 | 62 | 61 | 64 |
| asulox | 61 | 59 | 54 | 59 | 58 | 60 | 59 |
| tramat | 56 | 57 | 51 | 52 | 55 | 57 | 55 |
| tramat/asulox | 40 | 39 | 41 | 40 | 46 | 46 | 42 |

## P.rhoeas

| Young leaf | 1 | 2 | 3 | 4 | 5 | 6 | mean |
|---|---|---|---|---|---|---|---|
| ***water*** | | | | | | | |
| control | 128 | 130 | 128 | 122 | 124 | 126 | 126 |
| asulox | 134 | 128 | 129 | 129 | 134 | 131 | 131 |
| tramat | 128 | 130 | 127 | 129 | 126 | 129 | 128 |
| tramat/asulox | 124 | 125 | 128 | 126 | 121 | 122 | 124 |
| ***diquat*** | | | | | | | |
| control | 127 | 129 | 126 | 128 | 131 | 128 | 128 |
| asulox | 126 | 130 | 121 | 131 | 127 | 129 | 127 |
| tramat | 122 | 126 | 125 | 124 | 129 | 122 | 125 |
| tramat/asulox | 126 | 125 | 120 | 122 | 131 | 124 | 125 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 66 | 62 | 59 | 61 | 60 | 58 | 61 |
| asulox | 60 | 65 | 58 | 62 | 60 | 60 | 61 |
| tramat | 60 | 64 | 62 | 63 | 60 | 64 | 62 |
| tramat/asulox | 69 | 69 | 67 | 63 | 66 | 68 | 67 |

| old leaf | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***water*** | | | | | | | |
| control | 126 | 125 | 125 | 125 | 130 | 124 | 126 |
| asulox | 133 | 128 | 129 | 130 | 136 | 134 | 132 |
| tramat | 129 | 122 | 126 | 124 | 125 | 122 | 125 |
| tramat/asulox | 126 | 131 | 129 | 130 | 124 | 128 | 128 |
| ***diquat*** | | | | | | | |
| control | 133 | 133 | 135 | 130 | 132 | 131 | 132 |
| asulox | 129 | 126 | 131 | 130 | 128 | 122 | 128 |
| tramat | 127 | 126 | 122 | 129 | 128 | 128 | 127 |
| tramat/asulox | 122 | 124 | 128 | 126 | 131 | 129 | 127 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 56 | 55 | 59 | 52 | 56 | 52 | 55 |
| asulox | 55 | 56 | 56 | 62 | 57 | 60 | 58 |
| tramat | 54 | 55 | 58 | 56 | 50 | 52 | 54 |
| tramat/asulox | 44 | 40 | 47 | 46 | 42 | 46 | 44 |

## FUMITORY

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Young leaf | | | | | | | |
| ***water*** | | | | | | | |
| control | 130 | 136 | 132 | 132 | 131 | 129 | 132 |
| asulox | 133 | 134 | 132 | 131 | 132 | 130 | 132 |
| tramat | 110 | 109 | 113 | 112 | 111 | 113 | 111 |
| tramat/asulox | 98 | 101 | 105 | 102 | 108 | 100 | 102 |
| ***diquat*** | | | | | | | |
| control | 126 | 128 | 126 | 129 | 132 | 130 | 129 |
| asulox | 134 | 130 | 132 | 130 | 128 | 128 | 130 |
| tramat | 106 | 102 | 104 | 110 | 108 | 110 | 107 |
| tramat/asulox | 99 | 95 | 101 | 100 | 102 | 104 | 100 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 64 | 60 | 64 | 60 | 63 | 60 | 62 |
| asulox | 59 | 60 | 62 | 61 | 58 | 62 | 60 |
| tramat | 37 | 37 | 39 | 34 | 35 | 37 | 37 |
| tramat/asulox | 40 | 42 | 38 | 38 | 37 | 44 | 40 |
| old leaf | | | | | | | |
| ***water*** | | | | | | | |
| control | 127 | 129 | 125 | 122 | 125 | 124 | 125 |
| asulox | 125 | 125 | 128 | 132 | 124 | 129 | 127 |
| tramat | 123 | 124 | 127 | 124 | 126 | 128 | 125 |
| tramat/asulox | 125 | 128 | 127 | 130 | 128 | 128 | 128 |
| ***diquat*** | | | | | | | |
| control | 120 | 119 | 119 | 123 | 121 | 120 | 120 |
| asulox | 126 | 122 | 129 | 126 | 124 | 126 | 126 |
| tramat | 127 | 128 | 126 | 127 | 124 | 127 | 127 |
| tramat/asulox | 126 | 120 | 129 | 126 | 128 | 124 | 126 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 59 | 56 | 50 | 55 | 50 | 50 | 53 |
| asulox | 62 | 62 | 60 | 64 | 59 | 59 | 61 |
| tramat | 60 | 60 | 56 | 55 | 64 | 62 | 60 |
| tramat/asulox | 59 | 64 | 62 | 58 | 62 | 62 | 61 |

## Fat Hen

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Young leaf | | | | | | | |
| ***water*** | | | | | | | |
| control | 134 | 132 | 129 | 134 | 132 | 129 | 132 |
| asulox | 136 | 138 | 136 | 129 | 138 | 134 | 135 |
| tramat | 116 | 112 | 118 | 114 | 114 | 113 | 115 |
| tramat/asulox | 108 | 104 | 104 | 108 | 105 | 103 | 105 |
| ***diquat*** | | | | | | | |
| control | 126 | 130 | 129 | 132 | 128 | 129 | 129 |
| asulox | 129 | 132 | 130 | 130 | 129 | 130 | 130 |
| tramat | 116 | 110 | 108 | 109 | 110 | 112 | 111 |
| tramat/asulox | 104 | 102 | 106 | 106 | 106 | 102 | 104 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 54 | 55 | 56 | 52 | 56 | 52 | 54 |
| asulox | 55 | 59 | 52 | 54 | 56 | 55 | 55 |
| tramat | 42 | 42 | 38 | 39 | 40 | 40 | 40 |
| tramat/asulox | 38 | 40 | 42 | 40 | 40 | 39 | 40 |
| old leaf | | | | | | | |
| ***water*** | | | | | | | |
| control | 127 | 128 | 128 | 129 | 124 | 123 | 127 |
| asulox | 136 | 136 | 135 | 134 | 132 | 135 | 135 |
| tramat | 130 | 132 | 126 | 129 | 132 | 130 | 130 |
| tramat/asulox | 132 | 130 | 130 | 129 | 128 | 135 | 131 |
| ***diquat*** | | | | | | | |
| control | 134 | 135 | 130 | 132 | 132 | 130 | 132 |
| asulox | 134 | 131 | 130 | 132 | 132 | 129 | 131 |
| tramat | 132 | 135 | 128 | 129 | 133 | 134 | 132 |
| tramat/asulox | 130 | 130 | 130 | 134 | 136 | 133 | 132 |
| ***diq/d.-methyl*** | | | | | | | |
| control | 59 | 55 | 56 | 50 | 54 | 52 | 54 |
| asulox | 55 | 54 | 54 | 59 | 58 | 56 | 56 |
| tramat | 60 | 62 | 62 | 54 | 59 | 59 | 59 |
| tramat/asulox | 62 | 62 | 57 | 60 | 58 | 59 | 60 |

General Linear Models Procedure

Dependent Variable: ANGLE

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 31 | 653144.4595 | 21069.1761 | 406.98 | 0.0001 |
| Error | 543 | 28111.0188 | 51.7698 | | |
| Corrected Total | 574 | 681255.4783 | | | |

| R-Square | C.V. | Root MSE | ANGLE Mean |
|---|---|---|---|
| 0.958736 | 7.031864 | 7.195126 | 102.3217 |

General Linear Models Procedure

Dependent Variable: ANGLE

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 5 | 39.7510 | 7.9502 | 0.15 | 0.9789 |
| PLT | 3 | 3114.5469 | 1038.1823 | 20.05 | 0.0001 |
| AGE | 1 | 737.1971 | 737.1971 | 14.24 | 0.0002 |
| HERB | 2 | 636982.2531 | 318491.1266 | 6152.06 | 0.0001 |
| TRT | 3 | 9323.0297 | 3107.6766 | 60.03 | 0.0001 |
| AGE*HERB | 2 | 106.6444 | 53.3222 | 1.03 | 0.3577 |
| AGE*TRT | 3 | 2043.2037 | 681.0679 | 13.16 | 0.0001 |
| HERB*TRT | 6 | 474.1046 | 79.0174 | 1.53 | 0.1672 |
| AGE*HERB*TRT | 6 | 675.0174 | 112.5029 | 2.17 | 0.0441 |

# Appendix 4

**Poppy:**

| | young | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 2.83 | 2.83 |
| | | ii | 1.51 | 1.43 |
| | | iii | 1.35 | |
| | | | | % of control |
| | asulox | i | 2.58 | 91.17 |
| | | ii | 1.04 | 72.73 |
| | | iii | 1.47 | 102.80 |
| | tramat | i | 2.97 | 104.95 |
| | | ii | 1.12 | 78.32 |
| | | iii | 1.40 | 97.90 |
| | tramat/asulox | i | 2.52 | 89.05 |
| | | ii | 1.21 | 84.62 |
| | | iii | 1.40 | 97.60 |

| | old | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 1.86 | 1.86 |
| | | ii | 1.25 | 1.25 |
| | | iii | 1.25 | |
| | | | | % of control |
| | asulox | i | 1.16 | 62.37 |
| | | ii | 1.29 | 103.20 |
| | | iii | 1.00 | 80.00 |
| | tramat | i | 1.56 | 83.87 |
| | | ii | 0.84 | 76.80 |
| | | iii | 0.96 | 67.20 |
| | tramat/asulox | i | 1.51 | 81.18 |
| | | ii | 0.88 | 70.40 |
| | | iii | 0.97 | 77.60 |

**P.rhoeas**

| | young | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 2.01 | 2.01 |
| | | ii | 1.12 | 1.26 |
| | | iii | 1.40 | |
| | | | | % of control |
| | asulox | i | 2.30 | 114.43 |
| | | ii | 1.27 | 100.79 |
| | | iii | 1.47 | 116.67 |
| | tramat | i | 2.03 | 101.00 |
| | | ii | 1.64 | 130.16 |
| | | iii | 0.38 | 30.16 ** |
| | tramat/asulox | i | 2.03 | 101.00 |
| | | ii | 1.39 | 110.32 |
| | | iii | 1.17 | 92.86 |

| | old | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 1.88 | 1.88 |
| | | ii | 1.24 | 1.22 |
| | | iii | 1.19 | |
| | | | | % of control |
| | asulox | i | 1.84 | 97.87 |
| | | ii | 1.18 | 96.72 |
| | | iii | 1.18 | 96.72 |
| | tramat | i | 1.37 | 72.87 |
| | | ii | 1.05 | 86.07 |
| | | iii | 0.76 | 62.30 |
| | tramat/asulox | i | 1.32 | 70.21 |
| | | ii | 0.70 | 57.38 |
| | | iii | 0.92 | 75.41 |

## Fumitory:

| | young | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 8.75 | 8.75 |
| | | ii | 4.88 | 5.15 |
| | | iii | 5.41 | |
| | | | | % of control |
| | asulox | i | 8.29 | 94.74 |
| | | ii | 4.94 | 95.92 |
| | | iii | 5.83 | 113.20 |
| | tramat | i | 4.13 | 47.20 |
| | | ii | 2.16 | 41.94 |
| | | iii | 0.62 | 12.04 ** |
| | tramat/asulox | i | 3.76 | 42.97 |
| | | ii | 2.88 | 55.92 |
| | | iii | 2.20 | 42.72 |

| | old | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 3.33 | 3.33 |
| | | ii | 1.99 | 1.85 |
| | | iii | 1.70 | |
| | | | | % of control |
| | asulox | i | 2.68 | 80.48 |
| | | ii | 1.89 | 102.16 |
| | | iii | 1.68 | 90.81 |
| | tramat | i | 2.56 | 76.88 |
| | | ii | 1.69 | 91.35 |
| | | iii | 1.64 | 88.65 |
| | tramat/asulox | i | 2.62 | 78.68 |
| | | ii | 1.77 | 95.68 |
| | | iii | 1.62 | 87.57 |

## Fat hen

| | young | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 1.89 | 1.89 |
| | | ii | 0.89 | 0.86 |
| | | iii | 0.82 | |
| | | | | % of control |
| | asulox | i | 1.87 | 98.94 |
| | | ii | 0.75 | 87.21 |
| | | iii | 0.78 | 87.21 |
| | tramat | i | 1.36 | 71.96 |
| | | ii | 0.39 | 45.35 |
| | | iii | 0.55 | 63.95 |
| | tramat/asulox | i | 1.33 | 70.37 |
| | | ii | 0.66 | 76.74 |
| | | iii | 0.64 | 74.42 |

| | old | | wax units/100 mm2 | average |
|---|---|---|---|---|
| treatment | control | i | 1.19 | 1.19 |
| | | ii | 0.98 | 1.04 |
| | | iii | 1.10 | |
| | | | | % of control |
| | asulox | i | 1.10 | 92.44 |
| | | ii | 0.96 | 92.31 |
| | | iii | 0.89 | 85.58 |
| | tramat | i | 1.29 | 108.40 |
| | | ii | 0.66 | 63.46 |
| | | iii | 0.67 | 64.42 |
| | tramat/asulox | i | 1.31 | 110.42 |
| | | ii | 0.76 | 73.08 |
| | | iii | 0.73 | 70.19 |

** was repeated, but, still low.

General Linear Models Procedure

Dependent Variable: WAxes

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 25 | 21010.22601 | 840.40904 | 3.29 | 0.0002 |
| Error | 46 | 11750.20359 | 255.43921 | | |
| Corrected Total | | 71 | 32760.42960 | | |

| R-Square | C.V. | Root MSE | WA Mean |
|---|---|---|---|
| 0.641329 | 19.46984 | 15.98247 | 82.08833 |

General Linear Models Procedure

Dependent Variable: WAxes

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 2 | 653.952477 | 326.976239 | 1.28 | 0.2877 |
| PLT | 3 | 2265.005438 | 755.001813 | 2.96 | 0.0422 |
| AGE | 1 | 18.300974 | 18.300974 | 0.07 | 0.7902 |
| HERB | 2 | 5504.947541 | 2752.473771 | 10.78 | 0.0001 |
| PLT*AGE | 3 | 6251.477074 | 2083.825691 | 8.16 | 0.0002 |
| PLT*HERB | 6 | 2167.517429 | 361.252905 | 1.41 | 0.2297 |
| AGE*HERB | 2 | 986.289661 | 493.144830 | 1.93 | 0.1566 |
| PLT*AGE*HERB | 6 | 3028.133557 | 504.688926 | 1.98 | 0.0887 |

# Appendix 5

**Swansea**

## Poppy

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 13.51 | | 11.95 | | 8.68 | | 11.38 | |
| diq | 14.54 | 107.62 | 10.59 | 88.62 | 9.22 | 106.22 | 11.45 | 100.82 |
| diq + hoe | 10.62 | 78.61 | 10.14 | 84.85 | 8.36 | 96.31 | 9.71 | 86.59 |
| diq + EC | 10.08 | 74.61 | 9.65 | 80.75 | 8.50 | 97.93 | 9.41 | 84.43 |
| diq + new | 11.73 | 86.82 | 8.72 | 72.97 | 7.85 | 90.44 | 9.43 | 83.41 |
| diq + agral | 8.12 | 60.10 | 8.29 | 69.37 | 6.06 | 69.82 | 7.49 | 66.43 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 11.12 | | 13.10 | | 12.02 | | | |
| diq | 9.69 | 87.14 | 12.09 | 92.29 | 11.45 | 95.26 | 11.08 | 91.56 |
| diq + hoe | 10.41 | 93.62 | 9.73 | 74.27 | 9.77 | 81.28 | 9.97 | 83.08 |
| diq + EC | 9.97 | 89.68 | 9.58 | 73.13 | 7.21 | 59.98 | 8.92 | 74.26 |
| diq + new | 11.97 | 107.64 | 7.65 | 58.40 | 9.40 | 78.20 | 9.67 | 81.41 |
| diq + agral | 10.55 | 94.87 | 6.84 | 52.21 | 5.96 | 49.58 | 7.78 | 65.56 |

## Fat hen

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 4.77 | | 4.55 | | 4.87 | | 4.73 | |
| diq | 4.17 | 87.42 | 4.29 | 94.29 | 4.68 | 96.10 | 4.38 | 92.60 |
| diq + hoe | 3.59 | 75.26 | 4.01 | 88.13 | 4.39 | 90.14 | 4.00 | 84.51 |
| diq + EC | 3.69 | 77.36 | 3.75 | 82.42 | 3.40 | 69.82 | 3.61 | 76.53 |
| diq + new | 4.05 | 84.91 | 3.90 | 85.71 | 3.91 | 80.29 | 3.95 | 83.64 |
| diq + agral | 4.03 | 84.49 | 3.94 | 86.59 | 3.72 | 76.39 | 3.90 | 82.49 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 4.95 | | 6.60 | | 4.79 | | 5.45 | |
| diq | 4.70 | 94.95 | 4.78 | 72.42 | 4.68 | 97.70 | 4.72 | 88.36 |
| diq + hoe | 3.68 | 74.34 | 4.30 | 65.15 | 4.39 | 91.65 | 4.12 | 77.05 |
| diq + EC | 3.98 | 80.40 | 4.33 | 65.61 | 3.57 | 74.53 | 3.96 | 73.51 |
| diq + new | 3.67 | 74.14 | 3.94 | 59.70 | 4.19 | 87.47 | 3.93 | 73.77 |
| diq + agral | 4.21 | 85.05 | 4.16 | 63.03 | 3.91 | 81.63 | 4.09 | 76.57 |

## Fumitory

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 6.80 | | 5.18 | | 6.12 | | 6.03 | |
| diq | 4.92 | 72.35 | 4.95 | 95.56 | 4.54 | 74.18 | 4.80 | 80.70 |
| diq + hoe | 4.02 | 59.12 | 4.82 | 93.05 | 4.30 | 70.26 | 4.38 | 74.14 |
| diq + EC | 5.47 | 80.44 | 4.74 | 91.51 | 3.85 | 62.91 | 4.69 | 78.29 |
| diq + new | 5.63 | 82.79 | 4.75 | 91.70 | 3.59 | 58.66 | 4.66 | 77.72 |
| diq + agral | 4.98 | 73.24 | 3.53 | 68.15 | 4.05 | 66.18 | 4.19 | 69.19 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 7.01 | | 5.86 | | 10.70 | | 7.86 | |
| diq | 9.03 | 128.82 | 4.87 | 83.11 | 8.96 | 83.74 | 7.62 | 98.55 |
| diq + hoe | 4.07 | 58.06 | 3.79 | 64.68 | 4.81 | 44.95 | 4.22 | 55.90 |
| diq + EC | 4.17 | 59.49 | 5.28 | 90.10 | 4.28 | 40.00 | 4.58 | 63.20 |
| diq + new | 6.31 | 90.01 | 4.08 | 69.62 | 4.32 | 40.37 | 4.90 | 66.67 |
| diq + agral | 4.50 | 64.19 | 3.52 | 60.07 | 3.84 | 35.89 | 3.95 | 53.38 |

**Wesley Vale**

## Poppy

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 43.56 | | 65.45 | | 52.81 | | 53.94 | |
| diq | 42.08 | 96.56 | 41.39 | 63.24 | 33.98 | 64.34 | 39.14 | 74.71 |
| diq + hoe | 29.37 | 67.42 | 33.80 | 51.64 | 32.78 | 62.07 | 31.98 | 80.38 |
| diq + EC | 31.31 | 71.88 | 37.47 | 57.25 | 29.63 | 56.11 | 32.80 | 61.74 |
| diq + new | 26.90 | 61.75 | 26.89 | 41.08 | 21.27 | 40.28 | 25.02 | 47.71 |
| diq + agral | 30.89 | 70.91 | 31.67 | 48.39 | 30.82 | 58.36 | 31.13 | 59.22 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 52.52 | | 62.58 | | 59.53 | | 58.21 | |
| diq | 46.71 | 88.94 | 40.46 | 64.65 | 36.38 | 61.11 | 41.18 | 71.57 |
| diq + hoe | 30.75 | 58.55 | 41.64 | 66.54 | 37.55 | 63.08 | 36.65 | 62.72 |
| diq + EC | 34.55 | 65.78 | 34.82 | 55.64 | 35.15 | 59.05 | 34.84 | 60.16 |
| diq + new | 37.82 | 72.01 | 43.72 | 69.86 | 35.22 | 59.18 | 38.92 | 67.01 |
| diq + agral | 32.20 | 61.31 | 34.14 | 54.55 | 29.63 | 49.77 | 31.99 | 55.21 |

## Fat hen

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 3.46 | | 7.50 | | 10.26 | | 7.07 | |
| diq | 2.06 | 59.54 | 2.70 | 36.00 | 1.10 | 10.72 | 1.95 | 35.42 |
| diq + hoe | 1.13 | 32.66 | 1.08 | 14.40 | 1.52 | 14.81 | 1.24 | 20.62 |
| diq + EC | 1.43 | 41.33 | 1.39 | 16.53 | 1.14 | 11.11 | 1.32 | 23.66 |
| diq + new | 1.84 | 53.18 | 1.22 | 16.27 | 1.29 | 12.57 | 1.45 | 27.34 |
| diq + agral | 1.22 | 35.26 | 1.76 | 23.47 | 0.92 | 8.97 | 1.30 | 22.56 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 12.82 | | 14.09 | | 14.64 | | 13.85 | |
| diq | 2.77 | 21.61 | 2.96 | 21.01 | 1.10 | 7.51 | 2.28 | 16.71 |
| diq + hoe | 1.51 | 11.78 | 3.01 | 21.36 | 1.71 | 11.68 | 2.08 | 14.94 |
| diq + EC | 1.62 | 12.64 | 2.30 | 16.32 | 1.49 | 10.18 | 1.80 | 13.05 |
| diq + new | 1.41 | 11.00 | 1.41 | 10.01 | 2.52 | 17.21 | 1.78 | 12.74 |
| diq + agral | 1.85 | 14.43 | 1.65 | 11.71 | 1.41 | 9.63 | 1.64 | 11.92 |

## Fumitory

| + T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 28.91 | | 25.71 | | 30.46 | | 28.36 | |
| diq | 6.88 | 23.80 | 4.85 | 18.86 | 5.87 | 19.27 | 5.87 | 20.64 |
| diq + hoe | 3.57 | 12.35 | 3.95 | 15.36 | 3.81 | 12.51 | 3.78 | 13.41 |
| diq + EC | 4.88 | 16.88 | 6.41 | 24.93 | 5.86 | 19.24 | 5.72 | 20.35 |
| diq + new | 4.44 | 15.36 | 10.02 | 38.97 | 3.21 | 10.54 | 5.89 | 21.62 |
| diq + agral | 6.84 | 23.66 | 5.79 | 22.52 | 6.87 | 22.55 | 6.50 | 22.91 |

| - T/A | 1 weight | % control | 2 weight | % control | 3 weight | % control | mean weight | mean % |
|---|---|---|---|---|---|---|---|---|
| water | 29.34 | | 29.39 | | 35.11 | | 31.28 | |
| diq | 12.29 | 41.89 | 10.64 | 36.20 | 3.78 | 10.77 | 8.90 | 29.62 |
| diq + hoe | 9.47 | 32.28 | 7.75 | 26.37 | 2.05 | 5.84 | 6.42 | 21.50 |
| diq + EC | 9.98 | 34.01 | 10.10 | 34.37 | 7.89 | 22.47 | 9.32 | 30.28 |
| diq + new | 6.99 | 23.82 | 6.82 | 23.21 | 7.70 | 21.93 | 7.17 | 22.99 |
| diq + agral | 6.22 | 21.20 | 7.31 | 24.87 | 8.09 | 23.04 | 7.21 | 23.04 |

## Swansea

Poppy

| + T/A | replicate 1 | 2 | 3 | mean | - T/A | replicate 1 | 2 | 3 | mean |
|---|---|---|---|---|---|---|---|---|---|
| water | 9 | 9 | 9 | 9.00 | | 9 | 9 | 9 | 9.00 |
| diq | 8 | 8 | 8 | 8.00 | | 8 | 8 | 8 | 8.00 |
| diq + hoe | 7 | 7 | 7 | 7.00 | | 7 | 7 | 7 | 7.00 |
| diq + EC | 6 | 7 | 7 | 6.67 | | 6 | 7 | 6 | 6.33 |
| diq +new | 7 | 6 | 7 | 6.67 | | 7 | 5 | 7 | 6.33 |
| diq + agral | 7 | 6 | 6 | 6.33 | | 7 | 7 | 6 | 6.67 |

Fumitory

| + T/A | 1 | 2 | 3 | | - T/A | 1 | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|
| water | 9 | 9 | 9 | 9.00 | | 9 | 9 | 9 | 9.00 |
| diq | 7 | 8 | 8 | 7.67 | | 8 | 8 | 8 | 8.00 |
| diq + hoe | 5 | 6 | 5 | 5.33 | | 6 | 6 | 6 | 6.00 |
| diq + EC | 5 | 6 | 5 | 5.33 | | 5 | 6 | 5 | 5.33 |
| diq +new | 7 | 5 | 5 | 5.67 | | 6 | 5 | 5 | 5.33 |
| diq + agral | 7 | 5 | 4 | 5.33 | | 7 | 5 | 5 | 5.67 |

Fat Hen

| + T/A | 1 | 2 | 3 | | - T/A | 1 | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|
| water | 9 | 9 | 9 | 9.00 | | 9 | 9 | 9 | 9.00 |
| diq | 7 | 8 | 8 | 7.67 | | 8 | 8 | 8 | 8.00 |
| diq + hoe | 5 | 6 | 5 | 5.33 | | 3 | 5 | 6 | 4.67 |
| diq + EC | 6 | 6 | 5 | 5.67 | | 5 | 6 | 5 | 5.33 |
| diq +new | 6 | 5 | 5 | 5.33 | | 6 | 5 | 4 | 5.00 |
| diq + agral | 7 | 8 | 4 | 6.33 | | 7 | 6 | 5 | 6.00 |

## Wesley Vale

Poppy

| + T/A | 1 | 2 | 3 | | - T/A | 1 | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|
| water | 9 | 9 | 9 | 9.00 | | 9 | 9 | 9 | 9.00 |
| diq | 8 | 8 | 8 | 8.00 | | 9 | 9 | 9 | 9.00 |
| diq + hoe | 8 | 7 | 7 | 7.33 | | 8 | 8 | 7 | 7.67 |
| diq + EC | 7 | 7 | 7 | 7.00 | | 8 | 8 | 7 | 7.67 |
| diq +new | 7 | 7 | 7 | 7.00 | | 7 | 8 | 8 | 7.67 |
| diq + agral | 8 | 7 | 8 | 7.67 | | 8 | 8 | 8 | 8.00 |

Fumitory

| + T/A | 1 | 2 | 3 | | - T/A | 1 | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|
| water | 8 | 8 | 8 | 8.00 | | 9 | 9 | 9 | 9.00 |
| diq | 5 | 5 | 4 | 4.67 | | 7 | 7 | 4 | 6.00 |
| diq + hoe | 4 | 3 | 3 | 3.33 | | 6 | 6 | 4 | 5.33 |
| diq + EC | 3 | 4 | 4 | 3.67 | | 5 | 6 | 5 | 5.33 |
| diq +new | 4 | 3 | 4 | 3.67 | | 5 | 5 | 5 | 5.00 |
| diq + agral | 5 | 4 | 5 | 4.67 | | 6 | 5 | 6 | 5.67 |

Fat hen

| + T/A | 1 | 2 | 3 | | - T/A | 1 | 2 | 3 | |
|---|---|---|---|---|---|---|---|---|---|
| water | 8 | 8 | 8 | 8.00 | | 9 | 9 | 9 | 9.00 |
| diq | 7 | 5 | 5 | 5.67 | | 7 | 6 | 5 | 6.00 |
| diq + hoe | 4 | 3 | 4 | 3.67 | | 6 | 6 | 6 | 6.00 |
| diq + EC | 4 | 4 | 5 | 4.33 | | 5 | 6 | 5 | 5.33 |
| diq +new | 4 | 4 | 4 | 4.00 | | 5 | 5 | 5 | 5.00 |
| diq + agral | 4 | 5 | 3 | 4.00 | | 6 | 5 | 4 | 5.00 |

# Weslay Vale Experiment Results sheet

calculated

| Sample Number | Rep | Treat | Species | No Plants | FW (g) | Surf area MM2 | SA/FW | Water extract mls | Water extract abs 550 | 5% actetic acid mls | abs 55(left | Colour | Water extract Concn | Water extract Concn*vol (spray vol) | Water extract Retention on area basis (mls/cm2) | Water extract Retention (*100000) | Water extract Retention on FW basis | Acid extract Concn | Acid extract Concn*vol (spray vol) | Acid extract Retention on area (mls/cm2) | Acid extract Retention (*100000) | Acid extract Retention on FW basis | Total extract Amount | Total extract Retention on area (mls/cm2) | Total extract Retention (*100000) | Total extract Retention on FW basis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 | 1 -TA | Fat-hen | 5 | 1.53 | 3292 | 2152 | 37 | 0.059 | 25 | 0.038 | 1 | 1.31E-04 | 4.84E-03 | 1.47E-04 | 14.7 | 3.16E-03 | 8.31E-05 | 2.08E-03 | 6.31E-05 | 6.3 | 1.36E-03 | 6.92E-03 | 2.10E-04 | 21.0 | 4.52E-03 |
| 41 | 2 | 1 -TA | Fat-hen | 5 | 0.99 | 3105 | 3136 | 28 | 0.09 | 25 | 0.003 | 1 | 1.96E-04 | 5.49E-03 | 1.77E-04 | 17.7 | 5.55E-03 | 4.60E-06 | 1.15E-04 | 3.71E-06 | 0.4 | 1.16E-04 | 5.61E-03 | 1.81E-04 | 18.1 | 5.66E-03 |
| 77 | 3 | 1 -TA | Fat-hen | 5 | 2.93 | 6004 | 2049 | 36.5 | 0.218 | 25 | 0.016 | 1 | 4.66E-04 | 1.70E-02 | 2.84E-04 | 28.4 | 5.81E-03 | 3.38E-05 | 8.44E-04 | 1.41E-05 | 1.4 | 2.88E-04 | 1.79E-02 | 2.98E-04 | 29.8 | 6.10E-03 |
| 2 | 1 | 1 +TA | Fat-hen | 5 | 1.73 | 3264 | 1887 | 27 | 0.094 | 25 | 0.051 | 2 | 2.05E-04 | 5.53E-03 | 1.69E-04 | 16.9 | 3.19E-03 | 1.12E-04 | 2.81E-03 | 8.60E-05 | 8.6 | 1.62E-03 | 8.33E-03 | 2.55E-04 | 25.5 | 4.82E-03 |
| 38 | 2 | 1 +TA | Fat-hen | 5 | 1.43 | 2912 | 2036 | 18 | 0.121 | 25 | 0.013 | 1 | 2.62E-04 | 4.71E-03 | 1.62E-04 | 16.2 | 3.29E-03 | 2.70E-05 | 6.76E-04 | 2.32E-05 | 2.3 | 4.73E-04 | 5.39E-03 | 1.85E-04 | 18.5 | 3.77E-03 |
| 74 | 3 | 1 +TA | Fat-hen | 5 | 1.83 | 3456 | 1889 | 44.5 | 0.089 | 25 | 0.01 | 1 | 1.94E-04 | 8.64E-03 | 2.50E-04 | 25.0 | 4.72E-03 | 2.03E-05 | 5.08E-04 | 1.47E-05 | 1.5 | 2.77E-04 | 9.14E-03 | 2.65E-04 | 26.5 | 5.00E-03 |
| 11 | 1 | 2 -TA | Fat-hen | 5 | 2.08 | 4050 | 1947 | 32 | 0.056 | 25 | 0.044 | 1 | 1.24E-04 | 3.98E-03 | 9.83E-05 | 9.8 | 1.91E-03 | 9.66E-05 | 2.41E-03 | 5.96E-05 | 6.0 | 1.16E-03 | 6.40E-03 | 1.58E-04 | 15.8 | 3.07E-03 |
| 47 | 2 | 2 -TA | Fat-hen | 5 | 1.89 | 3824 | 2023 | 32.5 | 0.061 | 25 | 0.017 | 2 | 1.35E-04 | 4.39E-03 | 1.15E-04 | 11.5 | 2.32E-03 | 3.60E-05 | 9.00E-04 | 2.35E-05 | 2.4 | 4.76E-04 | 5.29E-03 | 1.38E-04 | 13.8 | 2.80E-03 |
| 83 | 3 | 2 -TA | Fat-hen | 5 | 4.21 | 7994 | 1899 | 29 | 0.691 | 25 | 0.026 | 2 | 1.46E-03 | 4.25E-02 | 5.31E-04 | 53.1 | 1.01E-02 | 5.62E-05 | 1.40E-03 | 1.76E-05 | 1.8 | 3.34E-04 | 4.39E-02 | 5.49E-04 | 54.9 | 1.04E-02 |
| 8 | 1 | 2 +TA | Fat-hen | 5 | 2.54 | 4496 | 1770 | 41.5 | 0.07 | 25 | 0.035 | 3 | 1.54E-04 | 6.39E-03 | 1.42E-04 | 14.2 | 2.52E-03 | 7.64E-05 | 1.91E-03 | 4.25E-05 | 4.2 | 7.52E-04 | 8.30E-03 | 1.85E-04 | 18.5 | 3.27E-03 |
| 44 | 2 | 2 +TA | Fat-hen | 5 | 1.42 | 3600 | 2535 | 20 | 0.083 | 25 | 0.005 | 2 | 1.81E-04 | 3.63E-03 | 1.01E-04 | 10.1 | 2.56E-03 | 9.09E-06 | 2.27E-04 | 6.31E-06 | 0.6 | 1.60E-04 | 3.86E-03 | 1.07E-04 | 10.7 | 2.72E-03 |
| 80 | 3 | 2 +TA | Fat-hen | 5 | 2.36 | 6048 | 2563 | 40 | 0.155 | 25 | 0.026 | 2 | 3.33E-04 | 1.33E-02 | 2.21E-04 | 22.1 | 5.65E-03 | 5.52E-05 | 1.40E-03 | 2.32E-05 | 2.3 | 5.95E-04 | 1.47E-02 | 2.44E-04 | 24.4 | 6.25E-03 |
| 17 | 1 | 3 -TA | Fat-hen | 5 | 2.30 | 4521 | 1966 | 31.5 | 0.088 | 25 | 0.047 | 1 | 1.92E-04 | 6.05E-03 | 1.34E-04 | 13.4 | 2.63E-03 | 1.03E-04 | 2.58E-03 | 5.71E-05 | 5.7 | 1.12E-03 | 8.63E-03 | 1.91E-04 | 19.1 | 3.75E-03 |
| 53 | 2 | 3 -TA | Fat-hen | 5 | 2.47 | 4844 | 1961 | 32 | 0.313 | 25 | 0.009 | 1 | 6.67E-04 | 2.13E-02 | 4.41E-04 | 44.1 | 8.64E-03 | 1.81E-05 | 4.51E-04 | 9.32E-06 | 0.9 | 1.83E-04 | 2.18E-02 | 4.50E-04 | 45.0 | 8.82E-03 |
| 89 | 3 | 3 -TA | Fat-hen | 5 | 1.85 | 3278 | 1772 | 42 | 0.391 | 25 | 0.006 | 1 | 8.32E-04 | 3.49E-02 | 1.07E-03 | 106.6 | 1.89E-02 | 1.13E-05 | 2.83E-04 | 8.64E-06 | 0.9 | 1.53E-04 | 3.52E-02 | 1.07E-03 | 107.4 | 1.90E-02 |
| 14 | 1 | 3 +TA | Fat-hen | 5 | 1.80 | 4276 | 2376 | 34 | 0.039 | 25 | 0.072 | 2 | 8.85E-05 | 3.01E-03 | 7.04E-05 | 7.0 | 1.67E-03 | 1.59E-04 | 3.98E-03 | 9.32E-05 | 9.3 | 2.21E-03 | 6.99E-03 | 1.64E-04 | 16.4 | 3.89E-03 |
| 50 | 2 | 3 +TA | Fat-hen | 5 | 1.92 | 3622 | 1886 | 26 | 0.206 | 25 | 0.02 | 1 | 4.41E-04 | 1.15E-02 | 3.17E-04 | 31.7 | 5.97E-03 | 4.27E-05 | 1.07E-03 | 2.95E-05 | 2.9 | 5.56E-04 | 1.25E-02 | 3.46E-04 | 34.6 | 6.53E-03 |
| 86 | 3 | 3 +TA | Fat-hen | 5 | 1.95 | 3581 | 1836 | 21.5 | 0.454 | 25 | 0.004 | 1 | 9.65E-04 | 2.07E-02 | 5.79E-04 | 57.9 | 1.06E-02 | 6.84E-06 | 1.71E-04 | 4.78E-06 | 0.5 | 8.78E-05 | 2.09E-02 | 5.84E-04 | 58.4 | 1.07E-02 |
| 23 | 1 | 4 -TA | Fat-hen | 5 | 1.63 | 3042 | 1866 | 23 | 0.038 | 25 | 0.035 | 2 | 8.64E-05 | 1.99E-03 | 6.53E-05 | 6.5 | 1.22E-03 | 7.64E-05 | 1.91E-03 | 6.28E-05 | 6.3 | 1.17E-03 | 3.90E-03 | 1.28E-04 | 12.8 | 2.39E-03 |
| 59 | 2 | 4 -TA | Fat-hen | 5 | 1.72 | 3644 | 2119 | 42 | 0.048 | 25 | 0.009 | 2 | 1.08E-04 | 4.52E-03 | 1.24E-04 | 12.4 | 2.63E-03 | 1.81E-05 | 4.51E-04 | 1.24E-05 | 1.2 | 2.62E-04 | 4.97E-03 | 1.36E-04 | 13.6 | 2.89E-03 |
| 95 | 3 | 4 -TA | Fat-hen | 5 | 2.26 | 4000 | 1770 | 44 | 0.136 | 25 | 0.001 | 2 | 2.93E-04 | 1.29E-02 | 3.23E-04 | 32.3 | 5.71E-03 | 1.17E-07 | 2.92E-06 | 7.29E-08 | 0.0 | 1.29E-06 | 1.29E-02 | 3.23E-04 | 32.3 | 5.71E-03 |
| 20 | 1 | 4 +TA | Fat-hen | 5 | 1.23 | 2153 | 1750 | 21.5 | 0.047 | 25 | 0.088 | 2 | 1.05E-04 | 2.27E-03 | 1.05E-04 | 10.5 | 1.84E-03 | 1.95E-04 | 4.88E-03 | 2.27E-04 | 22.7 | 3.97E-03 | 7.15E-03 | 3.32E-04 | 33.2 | 5.81E-03 |
| 56 | 2 | 4 +TA | Fat-hen | 5 | 1.39 | 2640 | 1899 | 17 | 0.067 | 25 | 0.011 | 2 | 1.48E-04 | 2.51E-03 | 9.51E-05 | 9.5 | 1.81E-03 | 2.25E-05 | 5.64E-04 | 2.13E-05 | 2.1 | 4.05E-04 | 3.07E-03 | 1.16E-04 | 11.6 | 2.21E-03 |
| 92 | 3 | 4 +TA | Fat-hen | 5 | 1.13 | 2315 | 2049 | 35 | 0.08 | 25 | 0.004 | 2 | 1.75E-04 | 6.13E-03 | 2.65E-04 | 26.5 | 5.42E-03 | 6.84E-06 | 1.71E-04 | 7.39E-06 | 0.7 | 1.51E-04 | 5.30E-03 | 2.72E-04 | 27.2 | 5.57E-03 |
| 29 | 1 | 5 -TA | Fat-hen | 5 | 2.27 | 5746 | 2531 | 34.5 | 0.073 | 25 | 0.052 | 1 | 1.60E-04 | 5.53E-03 | 9.62E-05 | 9.6 | 2.44E-03 | 1.14E-04 | 2.86E-03 | 4.98E-05 | 5.0 | 1.26E-03 | 8.39E-03 | 1.46E-04 | 14.6 | 3.70E-03 |
| 65 | 2 | 5 -TA | Fat-hen | 5 | 2.75 | 5357 | 1948 | 29 | 0.165 | 25 | 0.007 | 1 | 3.55E-04 | 1.03E-02 | 1.92E-04 | 19.2 | 3.74E-03 | 1.36E-05 | 3.39E-04 | 6.33E-06 | 0.6 | 1.23E-04 | 1.06E-02 | 1.98E-04 | 19.8 | 3.86E-03 |
| 101 | 3 | 5 -TA | Fat-hen | 5 | 1.86 | 3600 | 1935 | 33 | 0.137 | 25 | 0.007 | 2 | 2.95E-04 | 9.75E-03 | 2.71E-04 | 27.1 | 5.24E-03 | 1.36E-05 | 3.39E-04 | 9.43E-06 | 0.9 | 1.82E-04 | 1.01E-02 | 2.80E-04 | 28.0 | 5.42E-03 |
| 26 | 1 | 5 +TA | Fat-hen | 5 | 1.03 | 1990 | 1932 | 23.5 | 0.041 | 25 | 0.046 | 2 | 9.27E-05 | 2.16E-03 | 1.10E-04 | 11.0 | 2.12E-03 | 1.01E-04 | 2.53E-03 | 1.27E-04 | 12.7 | 2.45E-03 | 4.71E-03 | 2.36E-04 | 23.6 | 4.57E-03 |
| 62 | 2 | 5 +TA | Fat-hen | 5 | 2.17 | 3711 | 1710 | 24 | 0.143 | 25 | 0.001 | 1 | 3.08E-04 | 7.39E-03 | 1.99E-04 | 19.9 | 3.41E-03 | 1.17E-07 | 2.92E-06 | 7.86E-08 | 0.0 | 1.34E-06 | 7.40E-03 | 1.99E-04 | 19.9 | 3.41E-03 |
| 98 | 3 | 5 +TA | Fat-hen | 5 | 1.61 | 3154 | 1959 | 39.5 | 0.104 | 25 | 0.011 | 1 | 2.26E-04 | 8.92E-03 | 2.83E-04 | 28.3 | 5.54E-03 | 2.25E-05 | 5.64E-04 | 1.79E-05 | 1.8 | 3.50E-04 | 9.48E-03 | 3.01E-04 | 30.1 | 5.89E-03 |
| 35 | 1 | 6 -TA | Fat-hen | 5 | 1.75 | 3279 | 1874 | 38 | 0.034 | 25 | 0.059 | 1 | 7.80E-05 | 2.96E-03 | 9.04E-05 | 9.0 | 1.69E-03 | 1.30E-04 | 3.25E-03 | 9.93E-05 | 9.9 | 1.86E-03 | 6.22E-03 | 1.90E-04 | 19.0 | 3.55E-03 |
| 71 | 2 | 6 -TA | Fat-hen | 5 | 1.70 | 3429 | 2017 | 23 | 0.075 | 25 | 0 | 2 | 1.65E-04 | 3.78E-03 | 1.10E-04 | 11.0 | 2.23E-03 | -2.13E-06 | -5.32E-05 | -1.55E-06 | -0.2 | -3.13E-05 | 3.73E-03 | 1.09E-04 | 10.9 | 2.19E-03 |
| 107 | 3 | 6 -TA | Fat-hen | 5 | 1.90 | 4014 | 2113 | 18 | 0.269 | 25 | 0.015 | 2 | 5.74E-04 | 1.03E-02 | 2.57E-04 | 25.7 | 5.44E-03 | 3.15E-05 | 7.88E-04 | 1.96E-05 | 2.0 | 4.15E-04 | 1.11E-02 | 2.77E-04 | 27.7 | 5.85E-03 |
| 32 | 1 | 6 +TA | Fat-hen | 5 | 1.77 | 3258 | 1841 | 29 | 0.041 | 25 | 0.061 | 2 | 9.27E-05 | 2.69E-03 | 8.26E-05 | 8.3 | 1.52E-03 | 1.35E-04 | 3.37E-03 | 1.03E-04 | 10.3 | 1.90E-03 | 6.06E-03 | 1.86E-04 | 18.6 | 3.42E-03 |
| 68 | 2 | 6 +TA | Fat-hen | 4 | 1.70 | 3087 | 1816 | 32 | 0.081 | 25 | 0.003 | 2 | 1.77E-04 | 5.67E-03 | 1.84E-04 | 18.4 | 3.34E-03 | 4.60E-06 | 1.15E-04 | 3.73E-06 | 0.4 | 6.77E-05 | 5.79E-03 | 1.87E-04 | 18.7 | 3.40E-03 |
| 104 | 3 | 6 +TA | Fat-hen | 5 | 2.91 | 5391 | 1853 | 29.5 | 0.178 | 25 | 0.017 | 2 | 3.82E-04 | 1.13E-02 | 2.09E-04 | 20.9 | 3.87E-03 | 3.60E-05 | 9.00E-04 | 1.67E-05 | 1.7 | 3.09E-04 | 1.22E-02 | 2.26E-04 | 22.6 | 4.18E-03 |
| 6 | 1 | 1 -TA | Fumitory | 5 | 6.55 | 15400 | 2351 | 36 | 0.158 | 25 | 0.124 | 0 | 3.40E-04 | 1.22E-02 | 7.94E-05 | 7.9 | 1.87E-03 | 2.76E-04 | 6.90E-03 | 4.48E-05 | 4.5 | 1.05E-03 | 1.91E-02 | 1.24E-04 | 12.4 | 2.92E-03 |
| 42 | 2 | 1 -TA | Fumitory | 5 | 15.48 | 23190 | 1498 | 41 | 0.3 | 25 | 0.193 | 1 | 6.40E-04 | 2.62E-02 | 1.13E-04 | 11.3 | 1.69E-03 | 4.31E-04 | 1.08E-02 | 4.64E-05 | 4.6 | 6.96E-04 | 3.70E-02 | 1.60E-04 | 16.0 | 2.39E-03 |
| 78 | 3 | 1 -TA | Fumitory | 5 | 6.00 | 10520 | 1753 | 42 | 0.161 | 25 | 0.071 | 0 | 3.46E-04 | 1.45E-02 | 1.38E-04 | 13.8 | 2.42E-03 | 1.57E-04 | 3.93E-03 | 3.73E-05 | 3.7 | 6.55E-04 | 1.85E-02 | 1.76E-04 | 17.6 | 3.08E-03 |
| 3 | 1 | 1 +TA | Fumitory | 5 | 5.14 | 9052 | 1761 | 41.5 | 0.211 | 25 | 0.126 | 1 | 4.52E-04 | 1.87E-02 | 2.07E-04 | 20.7 | 3.65E-03 | 2.80E-04 | 7.01E-03 | 7.75E-05 | 7.7 | 1.36E-03 | 2.58E-02 | 2.85E-04 | 28.5 | 5.01E-03 |
| 39 | 2 | 1 +TA | Fumitory | 5 | 7.54 | 12800 | 1698 | 31 | 0.222 | 25 | 0.099 | 1 | 4.75E-04 | 1.47E-02 | 1.15E-04 | 11.5 | 1.95E-03 | 2.20E-04 | 5.50E-03 | 4.29E-05 | 4.3 | 7.29E-04 | 2.02E-02 | 1.58E-04 | 15.8 | 2.68E-03 |
| 75 | 3 | 1 +TA | Fumitory | 5 | 9.39 | 13370 | 1424 | 57 | 0.293 | 25 | 0.146 | 0 | 6.25E-04 | 3.56E-02 | 2.66E-04 | 26.6 | 3.79E-03 | 3.25E-04 | 8.13E-03 | 6.08E-05 | 6.1 | 8.66E-04 | 4.37E-02 | 3.27E-04 | 32.7 | 4.56E-03 |
| 12 | 1 | 2 -TA | Fumitory | 5 | 11.30 | 17250 | 1527 | 39.5 | 0.326 | 25 | 0.147 | 1 | 6.94E-04 | 2.74E-02 | 1.59E-04 | 15.9 | 2.43E-03 | 3.28E-04 | 8.19E-03 | 4.75E-05 | 4.7 | 7.25E-04 | 3.56E-02 | 2.06E-04 | 20.6 | 3.15E-03 |
| 48 | 2 | 2 -TA | Fumitory | 5 | 4.33 | 7140 | 1649 | 28 | 0.121 | 25 | 0.101 | 1 | 2.62E-04 | 7.33E-03 | 1.03E-04 | 10.3 | 1.69E-03 | 2.24E-04 | 5.61E-03 | 7.86E-05 | 7.9 | 1.30E-03 | 1.29E-02 | 1.81E-04 | 18.1 | 2.99E-03 |
| 84 | 3 | 2 -TA | Fumitory | 5 | 5.27 | 8646 | 1641 | 28 | 0.507 | 25 | 0.116 | 1 | 1.08E-03 | 3.01E-02 | 3.49E-04 | 34.9 | 5.72E-03 | 2.58E-04 | 6.45E-03 | 7.46E-05 | 7.5 | 1.22E-03 | 3.66E-02 | 4.23E-04 | 42.3 | 5.94E-03 |
| 9 | 1 | 2 +TA | Fumitory | 5 | 6.39 | 9450 | 1479 | 46 | 0.239 | 25 | 0.199 | 1 | 5.11E-04 | 2.35E-02 | 2.49E-04 | 24.9 | 3.68E-03 | 4.44E-04 | 1.11E-02 | 1.18E-04 | 11.8 | 1.74E-03 | 3.46E-02 | 3.66E-04 | 36.6 | 5.41E-03 |
| 45 | 2 | 2 +TA | Fumitory | 5 | 6.06 | 8680 | 1432 | 29 | 0.409 | 25 | 0.128 | 1 | 8.70E-04 | 2.52E-02 | 2.91E-04 | 29.1 | 4.16E-03 | 2.85E-04 | 7.12E-03 | 8.21E-05 | 8.2 | 1.15E-03 | 3.23E-02 | 3.73E-04 | 37.3 | 5.34E-03 |
| 81 | 3 | 2 +TA | Fumitory | 5 | 12.87 | 16000 | 1243 | 54.5 | 0.604 | 25 | 0.15 | 1 | 1.28E-03 | 8.25E-02 | 5.17E-04 | 51.7 | 6.42E-03 | 3.34E-04 | 8.36E-03 | 5.22E-05 | 5.2 | 6.49E-04 | 9.10E-02 | 5.69E-04 | 56.9 | 7.07E-03 |
| 18 | 1 | 3 -TA | Fumitory | 5 | 8.91 | 13780 | 1547 | 36.5 | 0.317 | 25 | 0.145 | 0 | 6.75E-04 | 2.47E-02 | 1.79E-04 | 17.9 | 2.77E-03 | 3.23E-04 | 8.08E-03 | 5.86E-05 | 5.9 | 9.06E-04 | 3.27E-02 | 2.38E-04 | 23.8 | 3.67E-03 |
| 54 | 2 | 3 -TA | Fumitory | 5 | 5.63 | 8650 | 1536 | 46.5 | 0.272 | 25 | 0.05 | 1 | 5.80E-04 | 2.70E-02 | 3.12E-04 | 31.2 | 4.79E-03 | 1.10E-04 | 2.75E-03 | 3.18E-05 | 3.2 | 4.88E-04 | 2.97E-02 | 3.44E-04 | 34.4 | 5.28E-03 |
| 90 | 3 | 3 -TA | Fumitory | 5 | 4.63 | 8110 | 1752 | 35 | 0.505 | 25 | 0.058 | 0 | 1.07E-03 | 3.75E-02 | 4.63E-04 | 46.3 | 8.11E-03 | 1.28E-04 | 3.20E-03 | 3.94E-05 | 3.9 | 6.91E-04 | 4.07E-02 | 5.02E-04 | 50.2 | 8.80E-03 |
| 15 | 1 | 3 +TA | Fumitory | 5 | 9.44 | 14480 | 1534 | 50 | 0.225 | 25 | 0.195 | 0 | 4.81E-04 | 2.41E-02 | 1.66E-04 | 16.6 | 2.55E-03 | 4.35E-04 | 1.09E-02 | 7.51E-05 | 7.5 | 1.15E-03 | 3.49E-02 | 2.41E-04 | 24.1 | 3.70E-03 |
| 51 | 2 | 3 +TA | Fumitory | 5 | 5.38 | 8650 | 1608 | 39.5 | 0.244 | 25 | 0.104 | 1 | 5.21E-04 | 2.06E-02 | 2.38E-04 | 23.8 | 3.83E-03 | 2.31E-04 | 5.78E-03 | 6.68E-05 | 6.7 | 1.07E-03 | 2.64E-02 | 3.05E-04 | 30.5 | 4.90E-03 |
| 87 | 3 | 3 +TA | Fumitory | 5 | 5.95 | 10000 | 1681 | 35 | 0.55 | 25 | 0.097 | 1 | 1.17E-03 | 4.09E-02 | 4.09E-04 | 40.9 | 6.87E-03 | 2.15E-04 | 5.39E-03 | 5.39E-05 | 5.4 | 9.05E-04 | 4.62E-02 | 4.62E-04 | 46.2 | 7.77E-03 |
| 24 | 1 | 4 -TA | Fumitory | 5 | 7.98 | 12460 | 1561 | 37 | 0.256 | 25 | 0.173 | 1 | 5.47E-04 | 2.02E-02 | 1.62E-04 | 16.2 | 2.53E-03 | 3.86E-04 | 9.65E-03 | 7.74E-05 | 7.7 | 1.21E-03 | 2.99E-02 | 2.40E-04 | 24.0 | 3.74E-03 |
| 60 | 2 | 4 -TA | Fumitory | 5 | 11.13 | 14800 | 1330 | 54 | 0.256 | 25 | 0.109 | 1 | 5.47E-04 | 2.95E-02 | 1.99E-04 | 19.9 | 2.65E-03 | 2.42E-04 | 6.06E-03 | 4.09E-05 | 4.1 | 5.44E-04 | 3.56E-02 | 2.40E-04 | 24.0 | 3.20E-03 |
| 96 | 3 | 4 -TA | Fumitory | 5 | 9.21 | 13314 | 1446 | 85 | 0.192 | 25 | 0.158 | 1 | 4.12E-04 | 3.50E-02 | 2.53E-04 | 26.3 | 3.80E-03 | 3.52E-04 | 8.81E-03 | 6.61E-05 | 6.6 | 9.56E-04 | 4.38E-02 | 3.29E-04 | 32.9 | 4.75E-03 |
| 21 | 1 | 4 +TA | Fumitory | 5 | 4.51 | 7444 | 1651 | 38.5 | 0.188 | 25 | 0.117 | 1 | 4.03E-04 | 1.55E-02 | 2.08E-04 | 20.8 | 3.44E-03 | 2.60E-04 | 6.51E-03 | 8.74E-05 | 8.7 | 1.44E-03 | 2.20E-02 | 2.96E-04 | 29.6 | 4.88E-03 |
| 57 | 2 | 4 +TA | Fumitory | 5 | 6.43 | 10400 | 1617 | 49 | 0.198 | 25 | 0.106 | 1 | 4.24E-04 | 2.08E-02 | 2.00E-04 | 20.0 | 3.23E-03 | 2.36E-04 | 5.89E-03 | 5.66E-05 | 5.7 | 9.16E-04 | 2.67E-02 | 2.56E-04 | 25.6 | 4.15E-03 |
| 93 | 3 | 4 +TA | Fumitory | 5 | 5.03 | 8560 | 1702 | 80.5 | 0.154 | 25 | 0.128 | 1 | 3.31E-04 | 2.57E-02 | 3.12E-04 | 31.2 | 5.30E-03 | 2.85E-04 | 7.12E-03 | 8.32E-05 | 8.3 | 1.42E-03 | 3.38E-02 | 3.95E-04 | 39.5 | 6.72E-03 |
| 30 | 1 | 5 -TA | Fumitory | 5 | 6.61 | 10360 | 1567 | 35.5 | 0.222 | 25 | 0.155 | 1 | 4.75E-04 | 1.69E-02 | 1.63E-04 | 16.3 | 2.55E-03 | 3.45E-04 | 3.54E-03 | 8.34E-05 | 8.3 | 1.31E-03 | 2.55E-02 | 2.46E-04 | 24.6 | 3.86E-03 |
| 66 | 2 | 5 -TA | Fumitory | 5 | 4.89 | 8120 | 1661 | 39 | 0.391 | 25 | 0.089 | 1 | 8.32E-04 | 3.24E-02 | 3.99E-04 | 39.9 | 6.63E-03 | 1.97E-04 | 4.94E-03 | 6.08E-05 | 6.1 | 1.01E-03 | 3.74E-02 | 4.60E-04 | 46.0 | 7.64E-03 |
| 102 | 3 | 5 -TA | Fumitory | 5 | 7.99 | 12081 | 1512 | 46.5 | 0.411 | 25 | 0.13 | 1 | 8.74E-04 | 4.06E-02 | 3.36E-04 | 33.6 | 5.09E-03 | 2.89E-04 | 7.24E-03 | 5.99E-05 | 6.0 | 9.06E-04 | 4.79E-02 | 3.96E-04 | 39.6 | 5.99E-03 |
| 27 | 1 | 5 +TA | Fumitory | 5 | 3.90 | 6001 | 1539 | 36 | 0.18 | 25 | 0.125 | 1 | 3.86E-04 | 1.39E-02 | 2.32E-04 | 23.2 | 3.56E-03 | 2.78E-04 | 6.96E-03 | 1.16E-04 | 11.6 | 1.78E-03 | 2.09E-02 | 3.48E-04 | 34.8 | 5.35E-03 |
| 63 | 2 | 5 +TA | Fumitory | 5 | 5.86 | 8404 | 1434 | 49 | 0.283 | 25 | 0.102 | 1 | 6.04E-04 | 2.96E-02 | 3.52E-04 | 35.2 | 5.05E-03 | 2.27E-04 | 5.67E-03 | 6.74E-05 | 6.7 | 9.67E-04 | 3.52E-02 | 4.19E-04 | 41.9 | 6.01E-03 |
| 99 | 3 | 5 +TA | Fumitory | 5 | 6.02 | 9463 | 1572 | 50 | 0.475 | 25 | 0.136 | 1 | 1.01E-03 | 5.04E-02 | 5.33E-04 | 53.3 | 8.38E-03 | 3.03E-04 | 7.57E-03 | 8.00E-05 | 8.0 | 1.26E-03 | 5.80E-02 | 6.13E-04 | 61.3 | 9.64E-03 |
| 36 | 1 | 6 -TA | Fumitory | 5 | 8.08 | 11400 | 1411 | 42 | 0.203 | 25 | 0.263 | 1 | 4.35E-04 | 1.83E-02 | 1.60E-04 | 16.0 | 2.26E-03 | 5.88E-04 | 1.47E-02 | 1.29E-04 | 12.9 | 1.82E-03 | 3.30E-02 | 2.89E-04 | 28.9 | 4.08E-03 |
| 72 | 2 | 6 -TA | Fumitory | 5 | 6.82 | 11320 | 1660 | 44 | 0.25 | 25 | 0.12 | 1 | 5.34E-04 | 2.35E-02 | 2.08E-04 | 20.8 | 3.44E-03 | 2.67E-04 | 6.67E-03 | 5.90E-05 | 5.9 | 9.79E-04 | 3.02E-02 | 2.67E-04 | 26.7 | 4.42E-03 |
| 108 | 3 | 6 -TA | Fumitory | 5 | 7.42 | 11150 | 1503 | 35 | 0.456 | 25 | 0.117 | 1 | 9.69E-04 | 3.39E-02 | 3.04E-04 | 30.4 | 4.57E-03 | 2.60E-04 | 6.51E-03 | 5.84E-05 | 5.8 | 8.77E-04 | 4.04E-02 | 3.52E-04 | 36.2 | 5.45E-03 |
| 33 | 1 | 6 +TA | Fumitory | 5 | 5.13 | 8750 | 1706 | 36.5 | 0.209 | 25 | 0.163 | 1 | 4.47E-04 | 1.63E-02 | 1.87E-04 | 18.7 | 3.18E-03 | 3.63E-04 | 9.09E-03 | 1.04E-04 | 10.4 | 1.77E-03 | 2.54E-02 | 2.90E-04 | 29.0 | 4.95E-03 |
| 69 | 2 | 6 +TA | Fumitory | 5 | 9.84 | 14440 | 1467 | 44 | 0.57 | 25 | 0.12 | 1 | 1.21E-03 | 5.32E-02 | 3.69E-04 | 36.9 | 5.41E-03 | 2.67E-04 | 6.67E-03 | 4.62E-05 | 4.6 | 6.78E-04 | 5.99E-02 | 4.15E-04 | 41.5 | 6.09E-03 |
| 105 | 3 | 6 +TA | Fumitory | 5 | 4.80 | 8090 | 1685 | 25.5 | 0.533 | 25 | 0.124 | 1 | 1.13E-03 | 2.89E-02 | 3.57E-04 | 35.7 | 6.01E-03 | 2.76E-04 | 6.90E-03 | 8.53E-05 | 8.5 | 1.44E-03 | 3.57E-02 | 4.42E-04 | 44.2 | 7.45E-03 |
| 4 | 1 | 1 -TA | Poppy | 5 | 11.70 | 29045 | 2482 | 39 | 0.227 | 50 | 0.087 | 1 | 4.85E-04 | 1.89E-02 | 6.52E-05 | 6.5 | 1.62E-03 | 1.93E-04 | 9.65E-03 | 3.32E-05 | 3.3 | 8.25E-04 | 2.86E-02 | 9.84E-05 | 9.8 | 2.44E-03 |
| 40 | 2 | 1 -TA | Poppy | 5 | 9.95 | 22246 | 2236 | 30.5 | 0.356 | 50 | 0.031 | 1 | 7.58E-04 | 2.31E-02 | 1.04E-04 | 10.4 | 2.32E-03 | 6.74E-05 | 3.37E-03 | 1.51E-05 | 1.5 | 3.39E-04 | 2.65E-02 | 1.19E-04 | 11.9 | 2.66E-03 |
| 76 | 3 | 1 -TA | Poppy | 5 | 11.33 | 24811 | 2190 | 36.5 | 0.321 | 50 | 0.018 | 1 | 6.84E-04 | 2.50E-02 | 1.01E-04 | 10.1 | 2.20E-03 | 3.82E-05 | 1.91E-03 | 7.71E-06 | 0.8 | 1.69E-04 | 2.59E-02 | 1.08E-04 | 10.8 | 2.37E-03 |
| 1 | 1 | 1 +TA | Poppy | 5 | 6.41 | 14914 | 2327 | 29.5 | 0.201 | 50 | 0.06 | 1 | 4.31E-04 | 1.27E-02 | 8.52E-05 | 8.5 | 1.98E-03 | 1.32E-04 | 6.62E-03 | 4.44E-05 | 4.4 | 1.03E-03 | 1.93E-02 | 1.30E-04 | 13.0 | 3.01E-03 |
| 37 | 2 | 1 +TA | Poppy | 5 | 12.51 | 28811 | 2303 | 30 | 0.334 | 50 | 0.031 | 1 | 7.11E-04 | 2.13E-02 | 7.41E-05 | 7.4 | 1.71E-03 | 6.74E-05 | 3.37E-03 | 1.17E-05 | 1.2 | 2.69E-04 | 2.47E-02 | 8.58E-05 | 8.6 | 1.98E-03 |
| 73 | 3 | 1 +TA | Poppy | 5 | 11.75 | 23731 | 2020 | 63 | 0.438 | 50 | 0.052 | 1 | 9.31E-04 | 5.86E-02 | 2.47E-04 | 24.7 | 4.99E-03 | 1.14E-04 | 5.72E-03 | 2.41E-05 | 2.4 | 4.87E-04 | 6.44E-02 | 2.71E-04 | 27.1 | 5.48E-03 |
| 10 | 1 | 2 -TA | Poppy | 5 | 8.74 | 19305 | 2209 | 46.5 | 0.265 | 50 | 0.075 | 2 | 5.66E-04 | 2.63E-02 | 1.36E-04 | 13.6 | 3.01E-03 | 1.66E-04 | 8.30E-03 | 4.30E-05 | 4.3 | 9.50E-04 | 3.46E-02 | 1.79E-04 | 17.9 | 3.96E-03 |
| 46 | 2 | 2 -TA | Poppy | 5 | 8.27 | 25858 | 3127 | 38.5 | 0.269 | 50 | 0.044 | 2 | 5.74E-04 | 2.21E-02 | 8.55E-05 | 8.5 | 2.67E-03 | 9.66E-05 | 4.83E-03 | 1.87E-05 | 1.9 | 5.84E-04 | 2.69E-02 | 1.04E-04 | 10.4 | 3.26E-03 |
| 82 | 3 | 2 -TA | Poppy | 5 | 9.41 | 21602 | 2296 | 67 | 0.515 | 50 | 0.049 | 2 | 1.09E-03 | 7.33E-02 | 3.39E-04 | 33.9 | 7.79E-03 | 1.08E-04 | 5.39E-03 | 2.49E-05 | 2.5 | 5.73E-04 | 7.86E-02 | 3.64E-04 | 36.4 | 8.36E-03 |
| 7 | 1 | 2 +TA | Poppy | 5 | 7.18 | 16127 | 2246 | 45.5 | 0.114 | 50 | 0.096 | 3 | 2.47E-04 | 1.12E-02 | 6.96E-05 | 7.0 | 1.56E-03 | 2.11E-04 | 1.05E-02 | 6.54E-05 | 6.5 | 1.47E-03 | 2.18E-02 | 1.35E-04 | 13.5 | 3.03E-03 |
| 43 | 2 | 2 +TA | Poppy | 5 | 10.81 | 22293 | 2062 | 49.5 | 0.421 | 50 | 0.05 | 2 | 8.95E-04 | 4.43E-02 | 1.99E-04 | 19.9 | 4.10E-03 | 1.10E-04 | 5.50E-03 | 2.47E-05 | 2.5 | 5.09E-04 | 4.98E-02 | 2.23E-04 | 22.3 | 4.61E-03 |
| 79 | 3 | 2 +TA | Poppy | 5 | 8.48 | 17700 | 2087 | 56.5 | 0.381 | 50 | 0.045 | 2 | 8.11E-04 | 4.58E-02 | 2.59E-04 | 25.9 | 5.40E-03 | 9.88E-05 | 4.94E-03 | 2.79E-05 | 2.8 | 5.83E-04 | 5.07E-02 | 2.87E-04 | 28.7 | 5.98E-03 |
| 16 | 1 | 3 -TA | Poppy | 5 | 9.00 | 21484 | 2387 | 50 | 0.209 | 50 | 0.072 | 1 | 4.47E-04 | 2.24E-02 | 1.04E-04 | 10.4 | 2.49E-03 | 1.59E-04 | 7.97E-03 | 3.71E-05 | 3.7 | 8.85E-04 | 3.03E-02 | 1.41E-04 | 14.1 | 3.37E-03 |
| 52 | 2 | 3 -TA | Poppy | 5 | 9.03 | 25000 | 2769 | 54 | 0.329 | 50 | 0.021 | 1 | 7.01E-04 | 3.78E-02 | 1.51E-04 | 15.1 | 4.19E-03 | 4.50E-05 | 2.25E-03 | 8.99E-06 | 0.9 | 2.49E-04 | 4.01E-02 | 1.60E-04 | 16.0 | 4.44E-03 |
| 88 | 3 | 3 -TA | Poppy | 5 | 11.64 | 25224 | 2167 | 78.5 | 0.502 | 50 | 0.018 | 1 | 1.07E-03 | 8.37E-02 | 3.32E-04 | 33.2 | 7.19E-03 | 3.82E-05 | 1.91E-03 | 7.58E-06 | 0.8 | 1.64E-04 | 8.56E-02 | 3.39E-04 | 33.9 | 7.35E-03 |
| 13 | 1 | 3 +TA | Poppy | 5 | 9.36 | 18750 | 2003 | 40 | 0.378 | 50 | 0.05 | 1 | 8.04E-04 | 3.22E-02 | 1.72E-04 | 17.2 | 3.44E-03 | 1.10E-04 | 5.50E-03 | 2.93E-05 | 2.9 | 5.88E-04 | 3.77E-02 | 2.01E-04 | 20.1 | 4.02E-03 |
| 49 | 2 | 3 +TA | Poppy | 5 | 7.97 | 15219 | 1910 | 38 | 0.697 | 50 | 0.035 | 2 | 1.48E-03 | 5.61E-02 | 3.69E-04 | 36.9 | 7.05E-03 | 7.64E-05 | 3.82E-03 | 2.51E-05 | 2.5 | 4.79E-04 | 6.00E-02 | 3.94E-04 | 39.4 | 7.52E-03 |
| 85 | 3 | 3 +TA | Poppy | 5 | 9.18 | 19456 | 2119 | 64 | 0.305 | 50 | 0.027 | 1 | 6.50E-04 | 4.16E-02 | 2.14E-04 | 21.4 | 4.53E-03 | 5.84E-05 | 2.92E-03 | 1.50E-05 | 1.5 | 3.18E-04 | 4.45E-02 | 2.29E-04 | 22.9 | 4.85E-03 |
| 22 | 1 | 4 -TA | Poppy | 5 | 9.05 | 21379 | 2362 | 43 | 0.254 | 50 | 0.066 | 2 | 5.42E-04 | 2.33E-02 | 1.09E-04 | 10.9 | 2.58E-03 | 1.46E-04 | 7.29E-03 | 3.41E-05 | 3.4 | 8.06E-04 | 3.06E-02 | 1.43E-04 | 14.3 | 3.38E-03 |
| 58 | 2 | 4 -TA | Poppy | 5 | 11.03 | 24582 | 2229 | 55 | 0.37 | 50 | 0.033 | 2 | 7.87E-04 | 4.33E-02 | 1.76E-04 | 17.6 | 3.93E-03 | 7.19E-05 | 3.59E-03 | 1.46E-05 | 1.5 | 3.26E-04 | 4.69E-02 | 1.91E-04 | 19.1 | 4.25E-03 |
| 94 | 3 | 4 -TA | Poppy | 5 | 10.17 | 20614 | 2027 | 67 | 0.363 | 50 | 0.025 | 1 | 7.73E-04 | 5.18E-02 | 2.51E-04 | 25.1 | 5.09E-03 | 5.39E-05 | 2.70E-03 | 1.31E-05 | 1.3 | 2.65E-04 | 5.45E-02 | 2.64E-04 | 26.4 | 5.35E-03 |
| 19 | 1 | 4 +TA | Poppy | 5 | 8.45 | 20060 | 2374 | 48 | 0.211 | 50 | 0.086 | 2 | 4.52E-04 | 2.17E-02 | 1.08E-04 | 10.8 | 2.57E-03 | 1.91E-04 | 9.54E-03 | 4.75E-05 | 4.8 | 1.13E-03 | 3.12E-02 | 1.56E-04 | 15.6 | 3.69E-03 |
| 55 | 2 | 4 +TA | Poppy | 5 | 7.93 | 15218 | 1919 | 28 | 0.412 | 50 | 0.045 | 1 | 8.76E-04 | 2.45E-02 | 1.61E-04 | 16.1 | 3.09E-03 | 9.88E-05 | 4.94E-03 | 3.25E-05 | 3.2 | 6.23E-04 | 2.95E-02 | 1.94E-04 | 19.4 | 3.72E-03 |
| 91 | 3 | 4 +TA | Poppy | 5 | 8.18 | 16685 | 2040 | 51 | 0.396 | 50 | 0.022 | 1 | 8.42E-04 | 4.30E-02 | 2.57E-04 | 25.7 | 5.25E-03 | 4.72E-05 | 2.36E-03 | 1.41E-05 | 1.4 | 2.89E-04 | 4.53E-02 | 2.72E-04 | 27.2 | 5.54E-03 |
| 28 | 1 | 5 -TA | Poppy | 5 | 6.22 | 14165 | 2277 | 49 | 0.172 | 50 | 0.086 | 2 | 3.69E-04 | 1.81E-02 | 1.28E-04 | 12.8 | 2.91E-03 | 1.91E-04 | 9.54E-03 | 6.73E-05 | 6.7 | 1.53E-03 | 2.76E-02 | 1.95E-04 | 19.5 | 4.44E-03 |
| 64 | 2 | 5 -TA | Poppy | 5 | 13.28 | 30864 | 2324 | 45 | 0.165 | 50 | 0.024 | 2 | 3.55E-04 | 1.60E-02 | 5.17E-05 | 5.2 | 1.20E-03 | 5.17E-05 | 2.58E-03 | 8.38E-06 | 0.8 | 1.95E-04 | 1.85E-02 | 6.01E-05 | 6.0 | 1.40E-03 |
| 100 | 3 | 5 -TA | Poppy | 5 | 10.40 | 21731 | 2090 | 48.5 | 0.293 | 50 | 0.024 | 2 | 6.25E-04 | 3.03E-02 | 1.39E-04 | 13.9 | 2.91E-03 | 5.17E-05 | 2.58E-03 | 1.19E-05 | 1.2 | 2.49E-04 | 3.29E-02 | 1.51E-04 | 15.1 | 3.16E-03 |
| 25 | 1 | 5 +TA | Poppy | 5 | 6.87 | 14781 | 2152 | 54 | 0.133 | 50 | 0.09 | 1 | 2.87E-04 | 1.55E-02 | 1.05E-04 | 10.5 | 2.26E-03 | 2.00E-04 | 9.99E-03 | 6.76E-05 | 6.8 | 1.45E-03 | 2.55E-02 | 1.72E-04 | 17.2 | 3.71E-03 |
| 61 | 2 | 5 +TA | Poppy | 5 | 9.03 | 17986 | 1992 | 39 | 0.499 | 50 | 0.024 | 2 | 1.06E-03 | 4.13E-02 | 2.30E-04 | 23.0 | 4.58E-03 | 5.17E-05 | 2.58E-03 | 1.44E-05 | 1.4 | 2.86E-04 | 4.39E-02 | 2.44E-04 | 24.4 | 4.86E-03 |
| 97 | 3 | 5 +TA | Poppy | 4 | 8.44 | 17669 | 2093 | 48 | 0.302 | 50 | 0.028 | 2 | 6.44E-04 | 3.09E-02 | 1.75E-04 | 17.5 | 3.66E-03 | 6.07E-05 | 3.03E-03 | 1.72E-05 | 1.7 | 3.59E-04 | 3.39E-02 | 1.92E-04 | 19.2 | 4.02E-03 |
| 34 | 1 | 6 -TA | Poppy | 5 | 12.02 | 27096 | 2254 | 50 | 0.274 | 50 | 0.105 | 2 | 5.85E-04 | 2.92E-02 | 1.08E-04 | 10.8 | 2.43E-03 | 2.33E-04 | 1.17E-02 | 4.31E-05 | 4.3 | 9.71E-04 | 4.09E-02 | 1.51E-04 | 15.1 | 3.40E-03 |
| 70 | 2 | 6 -TA | Poppy | 5 | 9.41 | 22430 | 2384 | 50.5 | 0.303 | 50 | 0.024 | 3 | 6.46E-04 | 3.26E-02 | 1.45E-04 | 14.5 | 3.47E-03 | 5.17E-05 | 2.58E-03 | 1.15E-05 | 1.2 | 2.75E-04 | 3.52E-02 | 1.57E-04 | 15.7 | 3.74E-03 |
| 106 | 3 | 6 -TA | Poppy | 5 | 10.19 | 22763 | 2234 | 34 | 0.486 | 50 | 0.036 | 3 | 1.03E-03 | 3.51E-02 | 1.54E-04 | 15.4 | 3.44E-03 | 7.86E-05 | 3.93E-03 | 1.73E-05 | 1.7 | 3.86E-04 | 3.90E-02 | 1.71E-04 | 17.1 | 3.83E-03 |
| 31 | 1 | 6 +TA | Poppy | 5 | 8.21 | 16513 | 2011 | 38 | 0.357 | 50 | 0.097 | 2 | 7.60E-04 | 2.89E-02 | 1.75E-04 | 17.5 | 3.52E-03 | 2.15E-04 | 1.08E-02 | 6.52E-05 | 6.5 | 1.31E-03 | 3.96E-02 | 2.40E-04 | 24.0 | 4.83E-03 |
| 67 | 2 | 6 +TA | Poppy | 5 | 11.49 | 25511 | 2220 | 55 | 0.381 | 50 | 0.043 | 2 | 8.11E-04 | 4.46E-02 | 1.75E-04 | 17.5 | 3.88E-03 | 9.48E-05 | 4.74E-03 | 1.86E-05 | 1.9 | 4.12E-04 | 4.93E-02 | 1.93E-04 | 19.3 | 4.29E-03 |
| 103 | 3 | 6 +TA | Poppy | 5 | 8.41 | 18038 | 2145 | 26 | 0.754 | 50 | 0.034 | 3 | 1.60E-03 | 1.06E-01 | 5.85E-04 | 58.5 | 1.26E-02 | 7.41E-05 | 3.71E-03 | 2.05E-05 | 2.1 | 4.41E-04 | 1.09E-01 | 6.06E-04 | 60.6 | 1.30E-02 |

**Swansea Trial**

General Linear Models Procedure

Dependent Variable: SCORE

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 25 | 61.34722222 | 2.45388889 | 13.59 | 0.0001 |
| Error | 46 | 8.30555556 | 0.18055556 | | |
| Corrected Total | | 71 | 69.65277778 | | |

| R-Square | C.V. | Root MSE | SCORE Mean |
|---|---|---|---|
| 0.880758 | 5.613599 | 0.424918 | 7.569444 |

General Linear Models Procedure

Dependent Variable: SCORE

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 2 | 0.36111111 | 0.18055556 | 1.00 | 0.3757 |
| PLT | 2 | 4.34027778 | 4.34027778 | 24.04 | 0.0001 |
| TA | 1 | 0.68055556 | 0.68055556 | 3.77 | 0.0583 |
| HERB | 5 | 44.90277778 | 8.98055556 | 49.74 | 0.0001 |
| PLT*TA | 1 | 1.68055556 | 1.68055556 | 9.31 | 0.0038 |
| PLT*HERB | 5 | 3.23611111 | 0.64722222 | 3.58 | 0.0081 |
| TA*HERB | 5 | 0.56944444 | 0.11388889 | 0.63 | 0.6772 |
| PLT*TA*HERB | 5 | 1.23611111 | 0.24722222 | 1.37 | 0.2533 |

General Linear Models Procedure

Dependent Variable: PERCENT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 25 | 19782.49693 | 791.29988 | 10.07 | 0.0001x |
| Error | 46 | 3613.05806 | 78.54474 | | |
| Corrected Total | | 71 | 23395.55499 | | |

| R-Square | C.V. | Root MSE | PERCENT Mean |
|---|---|---|---|
| 0.845566 | 11.52258 | 8.862547 | 76.91458 |

General Linear Models Procedure

Dependent Variable: PERCENT

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 2 | 1248.61021 | 624.30510 | 7.95 | 0.0011 |
| PLT | 2 | 2460.904055 | 2460.904055 | 31.33 | 0.0001 |
| TA | 1 | 3.03811 | 3.03811 | 0.04 | 0.8450 |
| HERB | 5 | 11167.46638 | 2233.49328 | 28.44 | 0.0001 |
| PLT*TA | 1 | 174.93851 | 174.93851 | 2.23 | 0.1424 |
| PLT*HERB | 5 | 1640.75295 | 328.15059 | 4.18 | 0.0033 |
| TA*HERB | 5 | 427.08875 | 85.41775 | 1.09 | 0.3800 |
| PLT*TA*HERB | 5 | 198.79391 | 39.75878 | 0.51 | 0.7700 |

**Wesley Vale Trial**

General Linear Models Procedure

Dependent Variable: SCORE

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 25 | 184.2916667 | 7.3716667 | 15.02 | 0.0001 |
| Error | 46 | 22.5833333 | 0.4909420 | | |
| Corrected Total | 71 | 206.8750000 | | | |

| R-Square | C.V. | Root MSE | SCORE Mean |
|---|---|---|---|
| 0.890836 | 11.75954 | 0.700673 | 5.958333 |

General Linear Models Procedure

Dependent Variable: SCORE

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 2 | 4.0833333 | 2.0416667 | 4.16 | 0.0219 |
| PLT | 2 | 9.5065666 | 9.5065666 | 38.73 | 0.0001 |
| TA | 1 | 8.6805556 | 8.6805556 | 17.68 | 0.0001 |
| HERB | 5 | 134.4583333 | 26.8916667 | 54.78 | 0.0001 |
| PLT*TA | 1 | 5.0138889 | 5.0138889 | 10.21 | 0.0025 |
| PLT*HERB | 5 | 10.5694444 | 2.1138889 | 4.31 | 0.0027 |
| TA*HER | 5 | 1.9027778 | 0.3805556 | 0.78 | 0.5727 |
| PLT*TA*HERB | 5 | 0.5694444 | 0.1138889 | 0.23 | 0.9466 |

General Linear Models Procedure

Dependent Variable: PERCENT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 25 | 71267.95833 | 2850.71833 | 28.26 | 0.0001 |
| Error | 46 | 4641.04826 | 100.89235 | | |
| Corrected Total | 71 | 75909.00659 | | | |

| R-Square | C.V. | Root MSE | PERCENT Mean |
|---|---|---|---|
| 0.938860 | 18.03473 | 10.04452 | 55.69542 |

General Linear Models Procedure

Dependent Variable: PERCENT

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| REP | 2 | 1904.19081 | 952.09540 | 9.44 | 0.0004 |
| PLT | 2 | 14639.585 | 14639.585 | 145.1 | 0.0001 |
| TA | 1 | 56.40990 | 56.40990 | 0.56 | 0.4584 |
| HERB | 5 | 30727.18848 | 6145.43770 | 60.91 | 0.0001 |
| PLT*TA | 1 | 764.86123 | 764.86123 | 7.58 | 0.0084 |
| PLT*HERB | 5 | 6660.06529 | 1332.01306 | 13.20 | 0.0001 |
| TA*HERB | 5 | 1363.15302 | 272.63060 | 2.70 | 0.0319 |
| PLT*TA*HERB | 5 | 512.91286 | 102.58257 | 1.02 | 0.4188 |

# Swansea Herbicide trial

Belmont (Ian Taylor)

**Treatments**

**Second sprays**

1 water (control)
2 Reglone/hoegrass
3 Reglone
4 Reglone/EC no active
5 Reglone/Agral
6 Reglone/Hoegrass new formulation

Split plot +/- Tramat Asulox
Three replications
Randomised complete block with split blocks

Spray -TA treatments with water
Rainwater used in all mixtures
Species Required: Fat hen
Fumitory
Poppies

10m

N

| | | |
|---|---|---|
| Rep 1 | 4 | +TA |
| | | -TA |
| | 6 | +TA |
| | | -TA |
| | 5 | +TA |
| | | -TA |
| | 2 | +TA |
| | | -TA |
| | 3 | +TA |
| | | -TA |
| | 1 | +TA |
| | | -TA |
| Rep 2 | 2 | +TA |
| | | -TA |
| | 4 | +TA |
| | | -TA |
| | 3 | +TA |
| | | -TA |
| | 5 | +TA |
| | | -TA |
| | 6 | +TA |
| | | -TA |
| | 1 | +TA |
| | | -TA |
| Rep 3 | 6 | +TA |
| | | -TA |
| | 3 | +TA |
| | | -TA |
| | 4 | +TA |
| | | -TA |
| | 5 | +TA |
| | | -TA |
| | 2 | +TA |
| | | -TA |
| | 1 | +TA |
| | 1 | -TA |

2m

24m

72

84m overall